## *The evolutionary and behavioural ecology of a European lamprey species pair (Lampetra fluviatilis and L. planeri): conservation concerns and anthropogenic impacts*

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# The evolutionary and behavioural ecology of a European lamprey species pair (*Lampetra fluviatilis* and *L. planeri*): conservation concerns and anthropogenic impacts

**Fiona Sheila Antoinette Bracken**

## Abstract

Lampreys (Order Petromyzontiformes) have existed for over 365 million years and are considered the most ancient group of living vertebrates. Given the socioeconomic, cultural, and ecological consequences of declining lamprey populations, it is imperative to address declines by implementing effective conservation management. This thesis explores the conservation issues affecting the European lamprey species pair *Lampetra fluviatilis* and *Lampetra planeri* and offers a holistic approach to their management and conservation in relation to anthropogenic impacts. The rapid development of small-scale hydropower provides substantial risk to migrating biota. At the site of an Archimedes screw turbine, damage rates to lampreys that passed through the screw were low (1.5%) and distinct seasonal, and diel, patterns of migration were exhibited by recently transformed juvenile and larval lampreys. Results indicated longer periods of impingement risk than expected. Cumulative potential impacts of multiple hydropower sites on downstream fish passage (including lampreys) should, however, be considered by regulatory agencies when planning hydropower development within catchments.

Anthropogenic barriers were also found to intensify differentiation between *L. planeri* populations and anadromous *L. fluviatilis* populations. Gene flow was consequently found to be asymmetric due to the barriers allowing downstream movement, whilst obstructing active upstream migration. Samples of 543 European river lamprey *Lampetra*

*fluviatilis* and European brook lamprey *Lampetra planeri* from across 15 sites, primarily in the British Isles, were investigated for 829bp mtDNA sequence and 13 polymorphic microsatellite DNA loci. Contrasting patterns of population structure were found for mtDNA (which revealed no differentiation between species) and microsatellite DNA markers. Microsatellite markers revealed strong differentiation among freshwater-resident *L. planeri* populations, and between *L. fluviatilis* and *L. planeri* in most cases, but little structure was evident among anadromous *L. fluviatilis* populations. There is also evidence that there has been some degree of gene flow between *L. fluviatilis* and *L. planeri* since these populations were established. There is much debate as to whether lamprey paired-species constitute distinct species or are divergent ecotypes of a single polymorphic species. Overall, these findings are suggestive of multiple independent divergences of *L. planeri* from an anadromous ancestor (i.e. *L. planeri* are polyphyletic). Focus of conservation and management efforts, therefore, needs to be directed towards ensuring the longitudinal connectivity within rivers, and the continued existence of the specific habitats necessitated within lamprey life-cycles. Molecular techniques should be applied to identify genetically differentiated populations of freshwater-resident lampreys. Appropriate measures, such as, the designation of a network of Special Areas of Conservation (SACs), and recognising these populations as distinct Evolutionarily Significant Units, should also be implemented to ensure the survival of these populations.

# The evolutionary and behavioural ecology of a European lamprey species pair (*Lampetra fluviatilis* and *L. planeri*): conservation concerns and anthropogenic impacts

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What would the world be, once bereft

Of wet and wilderness? Let them be left,

O let them be left, wilderness and wet;

Long live the weeds and the wilderness yet.

*Inversnaid, Gerard Manley Hopkins*

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Chapter Two has been accepted by River Research and Applications [Bracken FSA and Lucas MC (2013) Potential impacts of small-scale hydroelectric power generation on downstream moving lampreys. *River Research and Applications,* **29**, 1073-1081.] The chapter text contains most of the text within the accepted manuscript, with the inclusion of more detial in the thesis verioon.

**Declaration**

The material contained within this thesis has not previously been submitted for a degree at Durham University or any other university. The research reported here has been conducted by the author unless stated otherwise.

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**Dedication**

**In Loving Memory**

**of**

**Gerard Joseph Bracken**

**(1933-2010)**

Although you did not get the chance to see me complete this part of my life adventure, you were the one who set me out on this journey equipped with the tools to follow any dream I desired. You were a constant source of encouragement, and support, and proud of any endeavour I undertook. You were, and always will be, my hero and inspiration.

Go n-éirí an bóthar leat
Go raibh an ghaoth go brách ag do chúl
Go lonraí an ghrian go te ar d'aghaidh
Go dtite an bháisteach go mín ar do pháirceanna
Agus go mbuailimid le chéile arís,
Go gcoinní Dia i mbos A láimhe thú.

"The River," corrected the Rat. . . . It's my world, and I don't want any other. What it hasn't got is not worth having, and what it doesn't know is not worth knowing. Lord! the times we've had together! — *Kenneth Grahame,* The Wind in the Willows.

# Chapter 1 An introduction to lampreys: anthropogenic issues and implications for conservation

## 1.1 General introduction

Fishes are the most abundant vertebrates on the planet. With over 32,000 identified species, they exhibit greater diversity than any other group of vertebrates and can be found in nearly all aquatic environments, ranging from mountain streams to the abyssal depths of our oceans (Froese & Pauly 2013). Due to the lack of fur, feathers, or charisma (in contrast to fluffy, large eyed, mega-fauna) the public perception of fish is most commonly as the dead objects that appear on a plate in a restaurant or as a goldfish *Carassius auratus* in a bowl. However, there is growing interest in living fish due to the rising popularity of angling, having tropical aquaria in one's house, and a generally more positive attitude towards nature and the environment. The 2013 International Union for Conservation of Nature (IUCN) Red List names 2,110 fish species that are currently threatened with extinction (IUCN 2013). This figure was obtained using only the data available so far, which is limited to only 34% of the fish species that have been described to date. To put this in context, all of the 5,506 species of described mammals have been evaluated and 1,143 of those are listed as threatened in 2013 (IUCN 2013).

Freshwater habitats cover less than 1% of the world's surface, yet contain more than 126,000 known animal species (10,000 of these being fish), which includes over 25% of all described vertebrates, and approximately 2,600 macrophytes (Lundberg *et al.* 2000; IUCN 2013). Unfortunately, due to the small relative size of freshwater habitats, this makes the biodiversity that they support especially vulnerable to human activities and environmental change. Within Europe alone, more than one third (37%) of freshwater

fish species are threatened (Freyhof & Brooks 2011; Figure 1.1). Freshwater ecosystems provide many cultural, regulatory, economic, and supportive services that contribute both directly, and indirectly, to human wellbeing through a variety of means such as recreation, scenic values, power generation, drinking water, and maintenance of fisheries (Aylward *et al.* 2005). The livelihoods of many of the world's poorest communities are dependent on resources from freshwater ecosystems (Kent 1997). From a conservation perspective, the recognition that freshwater ecosystems contribute disproportionately to global biological richness is being eclipsed by the growing realisation that extinction risks in freshwaters could be among the greatest of all (Revenga *et al.* 2005; Strayer & Dudgeon 2010).

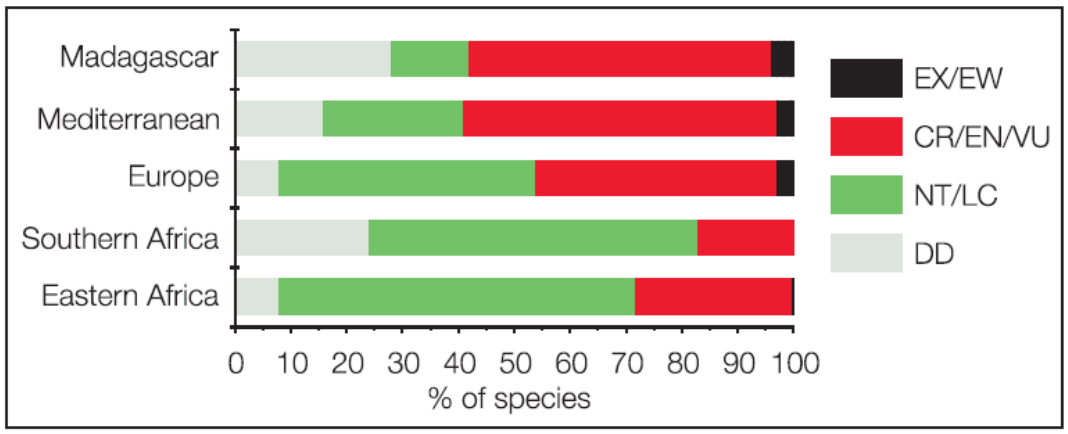



**Figure 1.1** The proportion of freshwater fish species in each IUCN threat category by geographic region. Abbreviations: EX/EW = Extinct/Extinct in the wild, CR/EN/VU = Critically Endangered/Endangered/Vulnerable, NT/LC = Near Threatened/Least Concern and DD = Data Deficient. Taken from the IUCN website (IUCN 2013).

The threats to global freshwater biodiversity can be grouped into five major categories, all of which have resulted in population declines and range reductions worldwide: overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by alien species (Allan & Flecker 1993; Malmqvist & Rundle 2002; Rahel 2002; Revenga *et al.* 2005; Palmer *et al.* 2008; Ormerod *et al.* 2010; Strayer &

Dudgeon 2010). In addition, climate change, increasing levels of water scarcity, and development goals such as increasing access to clean drinking water and sanitation, are all going to have major impacts upon freshwater systems in the future. Overall, this results in the decline in freshwater biodiversity, being far greater than in most terrestrial ecosystems (Sala *et al.* 2000).

The associated characteristics of discrete freshwater habitats render freshwater fishes especially vulnerable to threats (Table 1.1, Maitland 1995). A significant challenge to freshwater biodiversity conservation results from the complexity imposed on freshwater by catchment divides and both saltwater and anthropogenic barriers (Dudgeon *et al.* 2006). These can result in low rates of gene flow between populations, and consequently local adaptation, which can lead to considerable inter-catchment variation in biodiversity, and high levels of endemism rendering populations vulnerable to extinction due to their existence in a relatively small range. A fundamental starting point in trying to protect this biodiversity is to acquire basic knowledge about freshwater species, such as population size, habitat use, impacts of anthropogenic interference such as barriers to migration, pollutants, habitat loss, and assessing biodiversity within populations using molecular techniques. For the persistence of freshwater populations, and the maintenance of their integrity, it is, therefore, vital to identify Management Units (MUs), or Evolutionarily Significant Units (ESUs), within species and to have an understanding of the impacts of environmental and habitat change.

**Table 1.1** Some characteristics of freshwater fish populations which are relevant to their communities and conservation. Adapted from Maitland (1995).

| | |
|---|---|
| (1) Discreteness | Confined within their systems, independent populations arise leading to individual stock characteristics developing in isolation |
| (2) Numbers | Each population is often confined to a single (often small) aquatic system, within which there is usually significant water movement, which often leaves populations vulnerable to pollution, disease etc. Thus, for any species, the number of populations is of far greater importance than the number of individuals. |
| (3) Migrations | Many species of fish migrate as part of their life-cycles, during which they become especially vulnerable. In particular, diadromous (using freshwater and marine biotopes for life-cycle completion) and riverine species where the whole population must pass through the lower reaches of a river at least twice within their life-cycle. If the river is polluted, obstructed, or supports a large number of predators, entire populations are at risk of disappearing. |
| (4) Life Cycles | Large slow-growing species and small short-lived species are extremely vulnerable to fishing pressures and can be fished to extinction. |
| (5) Habitats | Being often confined to discrete systems, the life-cycle requirement for a species must be contained within that system. If something changes within the system which removes one of these requirements, populations can become vulnerable. |
| (6) Communities | Fish are typically key members of aquatic communities and food webs. Consequently, both fish populations and aquatic ecosystems can be disrupted by changes in habitat or the introduction of new species which are predators or competitors. |

Globally, awareness of the need to conserve freshwater biodiversity is limited. Between 1997 and 2001, only 7% of papers in the leading journal in the field, Conservation Biology, were concerned with freshwater species or habitats (Abell 2002). Research focus, and public awareness, of the threats to freshwater species need to be raised if conservation efforts are to succeed. In freshwater management, problems almost always involve simultaneous challenges, because human pressure typically alters more than one environmental factor (e.g. urbanisation affects runoff quantity, water quality, thermal

regimes, habitat availability, and the dispersal of invasive species), and also due to pressures from several sources often coinciding. This emphasises the importance of a multifaceted approach that not only examines the distribution, ecology and anthropogenic pressures that affect species today, but also takes into account the historical distribution and factors that have affected a species (or ecosystem) in the past.

Lampreys (Order Petromyzontiformes) have existed for over 365 million years and are considered the most ancient group of living vertebrates, comprising 42 extant species in three families; one in the Northern Hemisphere (Petromyzontidae) and two in the Southern Hemisphere (Geotriidae and Mordaciidae) with an anti-tropical distribution (Maitland & Campbell 1992; Potter & Gill 2003; Lang *et al.* 2009; Renaud 2011). There are three species of lamprey found in the British Isles: European river lamprey (*Lampetra fluviatilis* L., 1758), European brook lamprey (*Lampetra planeri* Bloch., 1784) and sea lamprey (*Petromyzon marinus* L., 1758) (Hardisty 1986a). All three species have a wide distribution in Europe (Figure 1.2), but populations of sea lamprey also extend across to eastern North America, Greenland and Iceland (Freyhof & Kottelat 2008a, b, c).

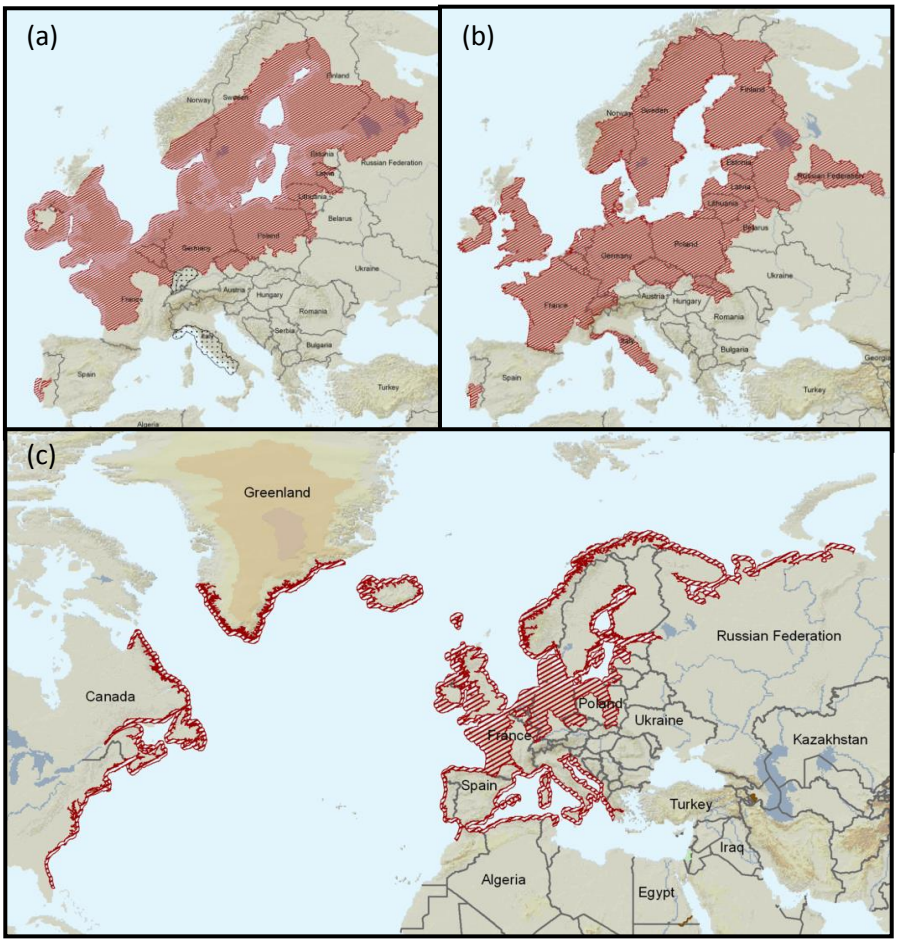



**Figure 1.2** Distribution of (a) *Lampetra fluviatilis* (b) *Lampetra planeri* and (c) *Petromyzon marinus*. Adapted from Freyhoff and Kottelat (2008a, b, c).

Over half of all lamprey species are considered to be endangered, vulnerable, or extinct in at least a portion of their range (Renaud 1997). Table 1.2 lists the current IUCN categories for each of the three species of lamprey present in Europe (within their natural range). *Petromyzon marinus*, *L. fluviatilis* and *L. planeri* are all listed under Annex II of the European Habitats Directive (92/43/EEC) as species whose conservation requires the designation of Special Areas of Conservation (SACs). *Lampetra fluviatilis* and *P. marinus* appear in Annex V, as species whose exploitation and taking in the wild may be subject to management measures (EC 1992). All three species are also listed in Appendix III of the Bern Convention, meaning, signatory countries are required to take ' appropriate and necessary legislative and administrative measures' to ensure their

protection (COE 1979). The factors affecting the conservation of lampreys are further discussed in Section 1.3.

Conversely, although the status of *P. marinus* is considered to be vulnerable in Europe (Renaud 1997), it has become an invasive pest in the Upper Laurentian Great Lakes (Smith & Tibbles 1980). On a global scale, the three species present in the British Isles are considered of '*Least Concern'* according to the IUCN Red List of Threatened Species (Freyhof & Kottelat 2008a, b, c) and the European Red List of Freshwater Fishes (Freyhof & Brooks 2011). Despite this categorisation, they are generally considered to be endangered within Europe (having become regionally extinct in Spain, Italy, Switzerland and Czech Republic, (Renaud 1997; Doadrio 2001; Lusk *et al.* 2004; Bianco & Delmastro 2011).

**Table 1.2** *Petromyzon marinus* and *Lampetra* spp. 2001 International Union for Conservation of Nature (IUCN) Red List categories for countries where information exists across their natural range. In Italy, *P. marinus* and *Lampetra fluviatilis* are often classified as Regionally Extinct, but these species still reproduce at least in the River Magra (Bianco & Delmastro 2011). In Slovenia, *P. marinus* is present in the Adriatic river basin (Povž 2011). In Lithuania, *L. fluviatilis* and *Lampetra planeri* are common, however, due to lack of data they have not been included in the Red data book (T. Virbickas & R. Repecka pers. comm.). RE = Regionally Extinct; CR= Critically Endangered; EN= Endangered; VU= Vulnerable; n/t = not threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. Other categories are R = Rare; NT = Near Threatened; LR = Lower Risk; NA = not applicable; X = species occurrence not confirmed; – = no data available/not included in the Red data book. Adapted from Mateus *et al.* (2012).

| | ***Petromyzon marinus*** | | ***Lampetra fluviatilis*** | | ***Lampetra planeri*** | |
|---|---|---|---|---|---|---|
| **Country** | **IUCN** | **Source** | **IUCN** | **Source** | **IUCN** | **Source** |
| **Russia** | EN | Russian Academy of Sciences ( 2001) | - | - | - | - |
| **Finland** | NA[a] | Rassi *et al.* (2010) | NT | Rassi *et al.* (2010), Urho & Lehtonen (2008) | LC | Kaukoranta *et al.* (2000) |
| **Norway** | LC[b] | Kålås *et al.* (2010) | LC[b] | Kålås *et al.* (2010) | LC[b] | Kålås *et al.*(2010) |
| **Sweden** | NT | Gärdenfors (2010) | LC | Gärdenfors (2010) | LC | Gärdenfors (2010) |
| **Estonia** | NE[c] | Lilleleht *et al.* (2008) | LC | Lilleleht *et al.* (2008) | DD | Lilleleht *et al.* (2008) |
| **Ireland** | VU | Maitland (2004) | LR | Maitland (2004) | LR | Maitland (2004) |
| **Great Britain** | VU | Maitland (2000) | VU | Maitland (2000) | VU | Maitland (2000) |
| **Denmark** | VU | Carl *et al.* (2004) | DD[d] | Carl *et al.* (2004) | LC | Carl *et al.* (2004) |
| **Lithuania** | EN[e] | Rašomavicius (2007) | - | - | - | - |
| **Poland** | EN | Głowacinski *et al.* (2002) | VU | Głowacinski *et al.* (2002) | VU | Witkowski *et al.* (2003) |
| **Belgium-Flanders** | RE | Kestemont (2010) | R | Kestemont (2010) | VU | Kestemont (2010) |
| **Belgium- Wallonia** | RE[f] | Philippart (2007), Kestemont (2010) | RE[f] | Philippart (2007), Kestemont (2010) | VU | Philippart (2007) Kestemont (2010) |
| **Germany** | n/t | Freyhof (2002) | n/t | Freyhof (2002) | n/t | Freyhof (2002) |
| **Czech Republic** | RE | Lusk *et al.* (2004) | RE | Lusk *et al.* (2004) | EN | Witkowski *et al.* (2003), Lusk *et al.* (2004) |
| **Ukraine** | X | X | - | - | LC | Witkowski *et al.* (2003) |
| **Slovakia** | - | - | X | X | CR | Witkowski *et al.* (2003) |
| **Switzerland** | - | - | RE | Kirchhofer *et al* .(2007) | EN | Kirchhofer *et al.* (2007) |
| **France** | NT | IUCN France *et al.* (2010) | VU | IUCN France *et al.* (2010) | LC | IUCN France *et al.* (2010) |
| **Slovenia** | EN[g] | Povž (2011) | X | X | - | - |
| **Croatia** | DD | Mrakovcic *et al.* (2007) | X | X | NT | Mrakovcic *et al.* (2007) |
| **Italy** | - | - | - | - | NT | Bianco & Delmastro (2011) |
| **Spain** | VU[h] | Doadrio (2001) | RE | Doadrio (2001) | CR[i] | Doadrio (2001) |
| **Portugal** | VU | Cabral *et al.* (2005) | CR | Cabral *et al.* (2005) | CR | Cabral *et al.* (2005) |

a = Recorded, but only occasionally and/or not reproducing; b = Little information available on the distribution and status in Norway. It is assumed that <1% of the total European stock occurs in Norway (E. Thorstad pers. comm.); c = Rare in Estonian waters. No reliable data available about the reproduction of sea lamprey in Estonia (Saat *et al.* 2002); d = Species is rare and may be threatened, but data are missing from several of the suspected habitats; therefore categorised as DD; e = Population abundance is very low, has been officially recorded in Lithuania a few times (T. Virbickas & R. Repecka pers. comm.); f = Likely to return (Philippart 2007); g = In Slovenia it is very rare and is restricted to the Pirano Bay and inflowing rivers in the North Adriatic Sea (Povž 2011); h = Endangered according to decree no. 139/2011 (BOE 2011), but only for populations from the Rivers Guadiana, Guadalquivir and Ebro and those from the southern basins; I = Vulnerable according to decree no. 139/2011 (BOE 2011).

This thesis aims to explore the conservation issues affecting *L. fluviatilis* and *L. planeri* in the British Isles with focus on concerns such as barriers, hydropower, evolutionary history and colonisation, and the identification of Evolutionarily Significant Units (ESUs) or Management Units (MUs). This will be achieved by using ecological methods to assess the impacts of hydropower on downstream moving lampreys, employing mitochondrial DNA markers to examine phylogeography and demographic history, the development, and the utilisation of microsatellite markers to explore population dynamics and identify ESUs. The remainder of this chapter will introduce the general conservation issues that affect lampreys and give a background that outlines their life-history and ecology.

## 1.2. Lamprey life- history

Lampreys are not the most aesthetically pleasing fish around, but are however, one of the most ancient and have been found in fossils dating back over 365 million years (Janvier *et al.* 2004; Gess *et al.* 2006). Lampreys, along with hagfish (Hyperotreti), belong to the superclass Agnatha and are classified as such by their lack of jaws. Lampreys can be identified by their eel-like bodies, cartilaginous skeleton, absence of scales, lack of lower jaws, and a mouth surrounded by a rasping sucker-like disc (Hardisty & Potter 1971b). Gill chambers open to seven holes on the outside of their body into which water is alternately drawn in and pumped out (Lewis 1980). Of the 42 described species of lamprey, 18 are parasitic as adults and typically display an anadromous life-history (such as *L. fluviatilis*), migrating between freshwater and marine or estuarine habitat to feed on host species as adults. This adult feeding phase is variable and can last from a few months to several years, after which lampreys return to freshwater to spawn and subsequently die. The 24 remaining species are commonly referred to as 'brook

lampreys' (such as *L. planeri*) and generally attain a smaller body size as adults. Brook lampreys are non-parasitic and do not feed as adults, remaining their whole lives in freshwater, making only relatively small migrations (ranging from 0-5 km) upstream to spawn (Hardisty 1944; Malmqvist 1980; Hume 2011).

All lamprey species spawn in running freshwater in a gravel/cobble substrate (Hardisty & Potter 1971b) They usually spawn in pairs or groups (i.e. polygamous mating) and will disperse their eggs in nests or shallow depressions in the bed material (Jang & Lucas 2005). *Lampetra planeri* and *L. fluviatilis* have a relatively low fecundity rate in comparison to *P. marinus,* which is also in proportion to the disparity in body sizes. *Lampetra fluviatilis* will produce between 11,000-26,000 oocytes per female, compared to 5,000-10,000 in *L. planeri* and 114,000-165,000 per female *P. marinus* (Hardisty 1970; Hardisty *et al.* 1970; Maitland 1980a). After a period of 15-30 days, the eggs hatch and develop into blind larvae known as ammocoetes (Figure 1.3), which swim/drift downstream and settle in a sand/silt substrate where they remain for roughly 3-7 years feeding on microscopic organisms filtered from the water (Maitland 2003). Ammocoetes may move both upstream and downstream as a result of either active movement or passive displacement to occupy new habitat, however, this behaviour is not well documented (Potter 1980; Maitland 2003; White & Harvey 2003; Quintella *et al.* 2004; Bracken & Lucas 2013).

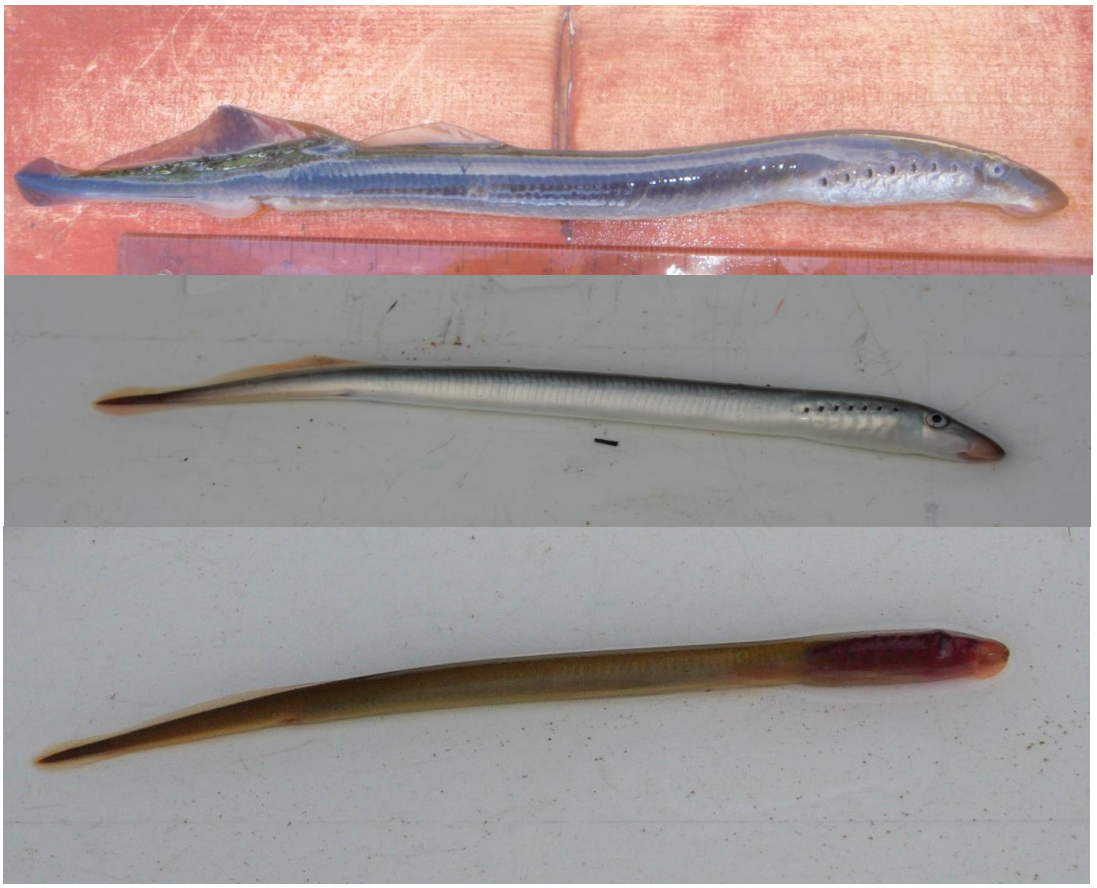


**Figure 1.3** The three different life stages of lampreys showing an adult female *Lampetra fluviatilis* (~30 cm) (top), *Lampetra fluviatilis* transformer (9.5 cm) (middle), and *Lampetra* spp. ammocoete (10 cm) (bottom).

The metamorphosis from larva to adult takes place over several months and in *L. fluviatilis*, changes occur that allow parastic feeding at this stage. During metamorphosis into what is known as the macrophthalmia (large–eyed), or transformer stage (Figure 1.3), individuals develop functional eyes and an oral disc, which in parasitic forms has sharpened teeth for feeding but is blunt in brook lampreys (Hardisty & Potter 1971a; Maitland 2003). A few months after the onset of transformation *L. fluviatilis* migrate to a marine, estuarine, (or in some populations lacustrine) environments to become ectoparasites to a variety of fish species for the next 1-2 years. *Lampetra fluviatilis*,

therefore, exhibit a diadromous (i.e. using freshwater and marine biotopes for life-cycle completion) life-cycle, which is interchanged with the term anadromous (i.e. migrating up rivers from the sea to spawn) throughout this thesis. Some large European lakes contain populations of *L. fluviatilis* known to feed exclusively in freshwater; including several lakes in Finland (Valovirta 1950; Tuunainen *et al.* 1980), Lough Neagh, Northern Ireland (Goodwin *et al.* 2006; Inger *et al.* 2010) and Loch Lomond, Scotland (Maitland 1980b; Adams *et al.* 2008). *Lampetra planeri*, however, does not feed after metamorphosis, the digestive tract becoming non-functional, and it differs in body size from the parasitic species (being nearly half the length) and remains in freshwater for the remainder of its life-cycle.

After spending roughly 18 months feeding in coastal waters, adult *L. fluviatilis* begin their upriver migration and cease feeding (Hardisty & Potter 1971b; Maitland 1980a). The time at which the spawning migration commences can vary widely, but usually occurs between September and December in the British Isles (Maitland 2003). In *L. fluviatilis*, some populations will begin their migration in spring (Maitland *et al.* 1994), some in autumn (Hardisty & Huggins 1973; Witkowski & Koszewski 1995), and others will do so throughout the winter (Sjöberg 1980; Hume 2011). *Lampetra planeri* populations will remain resident in freshwater throughout their lives and do not have a spawning migration in the same fashion as anadromous lampreys. *Lampetra planeri*, however, have been known to migrate between 2-5 km to spawning grounds both immediately prior to, or over a longer six month period before spawning (Hardisty 1944; Malmqvist 1980; Hume 2011). Migration usually occurs at night, however, lampreys exhibit additional diurnal activity during the spawning period through the loss of negative phototaxic behaviour, which results in twenty four hour locomotory activity (Sjöberg 1977; Jang & Lucas 2005). *Petromyzon marinus* is known to employ a mechanism of locating

conspecifics within rivers by means of following odours released from larval lamprey populations (Li *et al.* 1995). This increases the chances of finding suitable spawning habitat and potential mates at the end of their long and costly upriver migration. This pheromone mediated behaviour is also thought to exist in *L. fluviatilis* (Gaudron & Lucas 2006) and is discussed in more detail in Section 1.5.

For *L. fluviatilis* and *L .planeri,* spawning in the British Isles usually commences when water temperatures reach 10–11 ºC, usually around March or April (Morris & Maitland 1987). At the spawning sites there tends to be a male biased sex ratio in *L. planeri* populations (Hardisty 1961), however in *L. fluviatilis* the overall sex ratio is nearly equal (1 male: 1.2 females) which changes dramatically throughout the course of the spawning period from female dominated, during nest building and post-spawning within the nests, to male dominated during the time of active spawning (Jang & Lucas 2005). *Lampetra planeri* have been known to spawn within the same nests as *L. fluviatilis*, and sneaker male tactics have been identified in both *L. fluviatilis* and *L planeri* populations that could allow fertilisation despite the size differences between species (Lasne *et al.* 2010; Hume *et al.* 2013c).

In *P. marinus,* sexually mature males produce a pheromone that is highly attractive to sexually mature females, drawing them upstream to the spawning grounds and encouraging them to remain in the vicinity of the nests (Li *et al.* 2002; Li *et al.* 2003; Johnson *et al.* 2009; Johnson *et al.* 2012). Lampreys display nest-building behaviour as they reach the spawning grounds, moving large stones and gravel using their oral discs to create a depression in which to spawn (Jang & Lucas 2005). Typically within the depression, spawning usually commences with the male attaching to the

cephalic/branchial region of the female and wrapping the rest of his body around hers forming a loop. Once the tail loop is tightened, and ready to squeeze the eggs out of the female's body, both male and female will then thrash and vibrate their tails for several seconds, resulting in the expulsion of ova and milt (seminal fluid) into the gravel depression from where it is dispersed downstream with sand and silt particles by water currents (Applegate 1950). The number of eggs expressed in each spawning act is variable, but can be up to 100 in *L. fluviatilis* (Huggins & Thompson 1970). Spawning may last several days for each female but is dependent on the number of eggs available and numbers of eggs expressed during each spawning act. All lamprey species are semelparous, dying after a single spawning season (Larson 1980). Morbidity sets in quickly after spawning with *L. fluviatilis* moving into sheltered areas away from the main river flow, and *L. planeri* burrowing beneath stones while their bodies begin to break down and where they will eventually die (Hagelin 1959).

## 1.3 Conservation issues

The primary causes of species' declines, endangerments and extinctions, are anthropogenic (Lande 1998). As mentioned previously, the interacting influences of five major threats have been implicated the worldwide decline of freshwater biodiversity, including lampreys. These are: pollution, exploitation, flow modification, habitat degradation, and invasive species (Dudgeon *et al.* 2006). For example, excessive loading of nutrients and toxins in freshwater systems can cause eutrophication to the extent that they can no longer support their natural biotic communities (Smith 2003; Polunin 2008). Freshwater fishes are also seriously overexploited, leaving freshwater fisheries vulnerable to collapse and in global decline (Allan *et al.* 2005; Dudgeon *et al.* 2006). Non-native species have been introduced into freshwaters around the world, a considerable

number of which have had negative ecological impacts (such as outcompeting native biota for resources and introducing new pathogens), particularly in spatially restricted environments such as lakes (Dick *et al.* 1990; Kaufman 1992; Leppc *et al.* 2002; Strayer & Dudgeon 2010). River systems have been fragmented by *c.* 1 million dams globally, which limit habitat availability and can isolate populations (Nilsson *et al.* 2005). These impacts have a knock-on effect within freshwater ecosystems, and have ultimately resulted in population declines and range reductions of freshwater biodiversity worldwide (Dudgeon *et al.* 2006).

Marked declines in the abundance of lampreys can also be largely attributed to five major threats (Figure 1.4) as mentioned previously (Renaud 1997; Kelly & King 2001; Masters *et al.* 2006). For instance, larval lampreys (i.e. ammocoetes), are particularly vulnerable to pollution events because they spend multiple years in limited habitats (Moyle *et al.* 2009). Adult lampreys are also demonstrably susceptible to pollution, and it is likely that entire populations have been extirpated from rivers that became heavily polluted (Renaud 1997; Mateus *et al.* 2012). European river lampreys (*L. fluviatilis*) are still taken by commercial fisheries in many Swedish and Finnish rivers, and also fisheries within many rivers that drain into the Baltic Sea (Tuunainen *et al.* 1980; Valtonen 1980; Maitland & Campbell 1992; Ojutkangas *et al.* 1995; Masters *et al.* 2006; Sjöberg 2011), and the unregulated commercial exploitation of *L. fluviatilis* in the tidal River Ouse in England has also, in the past, threatened the species (Masters *et al.* 2006). The commercial exploitation of lampreys is further discussed in Section 1.3.1.

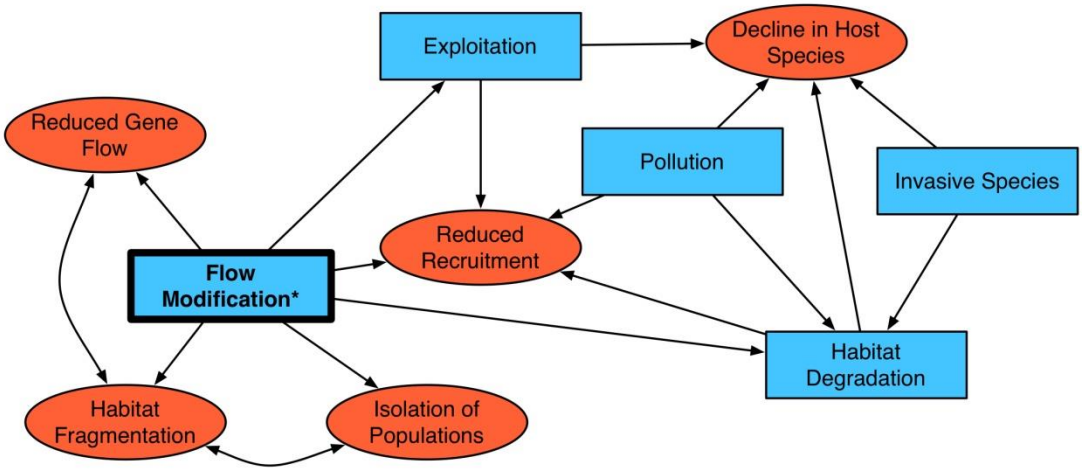



**Figure 1.4** Flow chart showing the possible interactions between the five main threats to freshwater species (blue rectangle), and some of the potential impacts on lamprey populations (red ovals). **Flow modification***, indicates that this threat will be examined in more detail within the thesis.

Similarly, it has also been suggested that the exploitation, or decline (by means of one of the other major threats to freshwater biodiversity) of the hosts on which lampreys feed has been a limiting factor for some lamprey populations (Birzaks & Abersons 2011; Murauskas *et al.* 2013). For example, the nationally rare whitefish *Coregonus lavaretus* is a known host of a freshwater-resident population *L. fluviatilis* within Loch Lomond, Scotland (Maitland 1980b). However, it is believed that the native population of *C. lavaretus* in Loch Lomond may be adversely affected by an invasive species (ruffe, *Gymnocephalus cernuus*) predating upon *C. lavaretus* eggs (Adams & Tippett 1991; Etheridge *et al.* 2011). A decline within the population of *C. lavaretus* could, therefore, adversely affect the native lamprey population by reducing the availability of host species on which adult lampreys may feed. Conversely, it has been suggested that, although *L. fluviatilis* may have altered its trophic feeding ecology in response to the negative impacts caused by the non-native species, these introduced species may actually help sustain lamprey populations by providing an alternative food source (Inger *et al.* 2010; Hume *et al.* 2013a).

River channelisation, habitat modifications, hydropower turbines, and artificial barriers have all adversely affected fish communities; however, anadromous species are particularly at risk (Armin 1998). Due to the life-history strategy of lampreys, there are a range of specific habitat needs for each life stage, which have been previously outlined in Section 1.2. Access to (for anadromous species in particular), the ability to locate, and the preservation of, these habitat types are consequently all critical factors in allowing lampreys to complete their life-cycle (discussed further in Section 1.3.2). Therefore, these issues (i.e. issues broadly relating to flow modification), which are a subset of the overall factors contributing to lamprey decline, will be considered within this thesis (Figure 1.4).

### 1.3.1 Commercial exploitation

Lampreys have been subject to a long history of exploitation within European rivers and are marketed either for human consumption (Tuunainen *et al.* 1980) or sport fishing bait (Masters *et al.* 2006). Anadromous lampreys are often intercepted during their spawning migration before they have had a chance to spawn (after which they will subsequently die) making them highly susceptible to population decline due to exploitation. *Lampetra fluviatilis* have in the past been exploited in Scandinavia (Sjöberg 1980), and also in Finnish coastal waters for human consumption from the early 1500s, with total annual lamprey catches estimated at between 2 to 2.5 million individuals (Tuunainen *et al.* 1980; Valtonen 1980; Sjöberg 2011). Polish rivers have had a *L. fluviatilis* fishery throughout the 20$^{th}$ century, but towards the end of the 1950s lamprey fishing ceased because catches fell to approximately 10kg per year (Witkowski 1992). In Portugal, the high economic value of *P. marinus* makes them the preferred target (*L. fluviatilis* are not abundant in Portugal and are considered to be critically endangered and, therefore,

probably not viable for exploitation) of both professional fishermen and poachers, creating a major threat to the their sustainability and conservation (Andrade *et al.* 2007).

In the past, commercial fishing of river lamprey within the UK has largely targeted populations found in the River Severn, River Thames, and the Trent and Ouse subcatchments of the Humber basin (Maitland 2003; Masters *et al.* 2006). In the late 19th and early 20th centuries, commercial fisheries operated in the Ouse, and Trent catchments of the Humber basin, targeting adult river lampreys during their autumn–winter spawning migration (Leaf 1908-1914; Spicer 1937) and selling the catch as bait for the North Sea long-line fishery. Lampreys were formerly a delicacy in Britain, to the extent that King Henry I of England reportedly died due to their overconsumption (Hollister 2003).

In recent years, river lampreys have again been caught in large numbers by an interceptive fishery in the tidal Ouse, and the catch sold to anglers for use as bait (Masters *et al.* 2006). Technically, lampreys were seen as by-catch in a licensed eel fishery; however, yields were such that it was essentially a commercial lamprey fishery (Masters *et al.* 2006). Consequently, targeted trapping of lampreys was not licensed and, therefore, was illegal. Eel traps, however, do require licensing, and eels can be fished throughout the year, meaning any by-catch of lampreys, regardless of scale, fall outside the jurisdiction of the regulating bodies (unless the condition of an SAC is threatened, in which case the appropriate authority would take action). The UK Marine and Coastal Access Act 2009 was an important step towards the careful regulation of lamprey exploitation. As of 1st January, 2011, fishing of *L. fluviatilis* within the UK requires authorisation which has restrictions on take (1044 kg per season) and timing (season

open from 1st November to 10th December). It is essential, however, that this is regulated effectively, and future enforcement of this legislation is imperative to ensure positive changes to the impact of commercial exploitation on lampreys.

### 1.3.2 Flow modification (barriers to migration and hydropower)

Anthropogenic barriers such as barrages, dams, and weirs can radically reduce the longitudinal connectivity of rivers and can alter the composition and availability of surrounding riverine habitat, which may also cause significant losses to the spawning and nursery habitat of many fluvial species (Renaud 1997; Nilsson *et al.* 2005). Restricting access to, or destroying, spawning habitat and capturing adult migrants before they have had the opportunity to spawn can render lamprey populations more vulnerable to extirpation (Masters *et al.* 2006). Mateus *et al.* (2012) indicate that on average, 80% of spawning habitat in the major river basins in the Iberian Peninsula used by anadromous *P. marinus* and *L. fluviatilis* is now unavailable due to the extensive construction of dams in the lower stretches of the river. Similarly, Lucas *et al.,* (2009) reveal that although 98% of spawning habitat was present above five low-head weirs (2-3 m high) in the River Derwent, north-east England, on average just 1.8 % of adult spawning *L. fluviatilis* were recorded there.

Lampreys are often sensitive to freshwater habitat alteration (Figure 1.5) and as a result most species have declined in distribution and abundance over recent decades and many species are now regarded as threatened (Renaud 1997; Baras & Lucas 2001; Nunn *et al.* 2008). Both adult and transformer stage lampreys (especially of anadromous species) are vulnerable to the effects of barriers to migration (Figure 1.5). Dams/weirs can obstruct and delay passage during the upstream migration of adult lampreys, and entrainment in

hydropower turbines or impingement on screens at hydropower facilities can injure or kill downstream migrating transformers (or drifting ammocoetes). Structures within rivers can also alter the surrounding habitat by means of changes to flow and hydrology, which can ultimately affect the composition and availability of sand/silt (nursery) and gravel habitats (spawning) required within the life-cycle of lampreys (Figure 1.5).

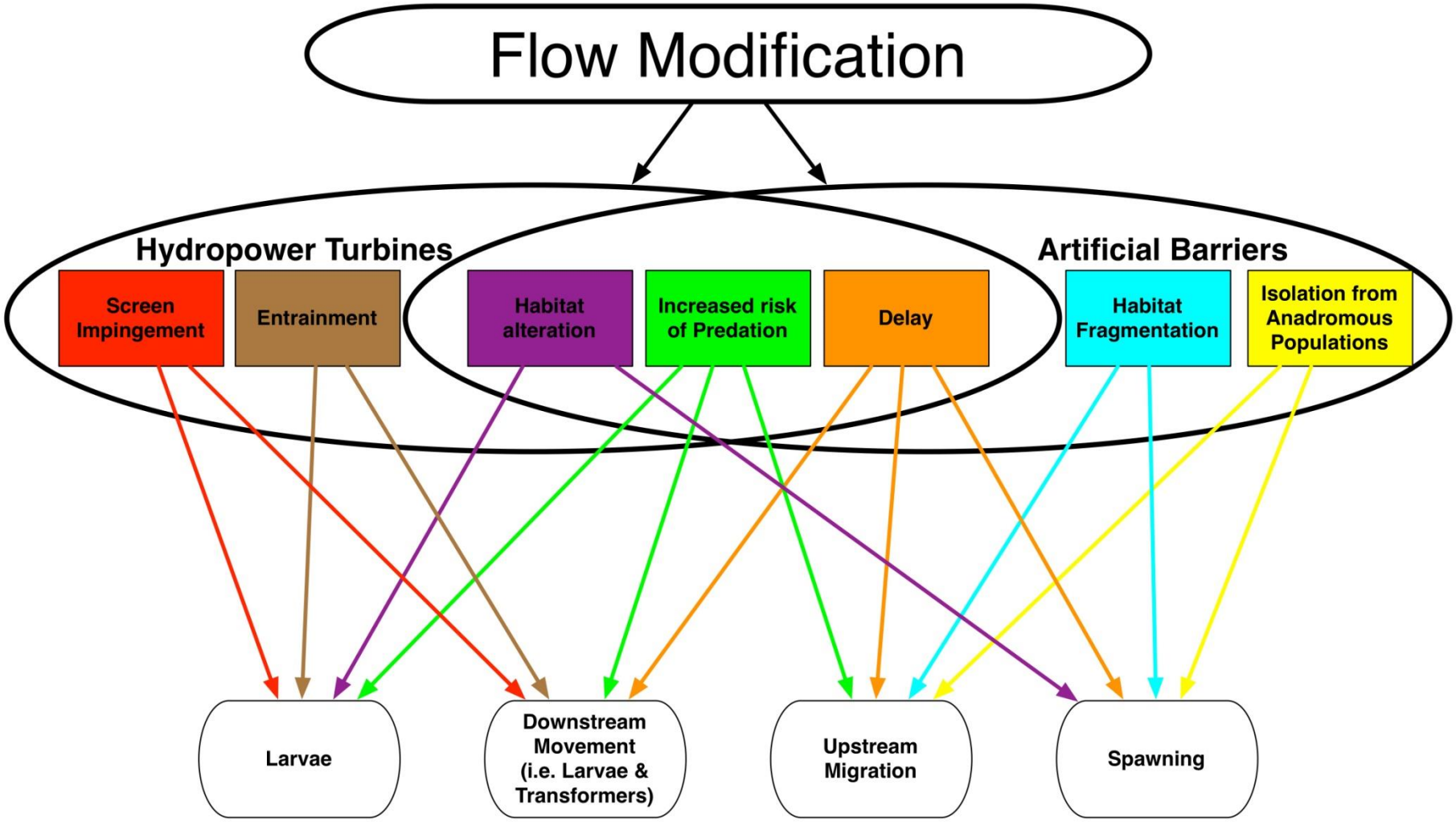



**Figure 1.5** Diagram illustrating the way in which flow modification can affect, or potentially affect, the differing life-history stages of lampreys.

Diadromous fish, such as lampreys, are more vulnerable than non-migratory freshwater species in many ways as they must exploit a diversity of habitats within distinct biomes, in which to live and complete their life-cycles. These habitats may be distantly spaced, requiring long distance migrations from one habitat to another, making their successful passage vital in completing their life-cycle (McDowall 1992; Amoros & Bornette 2002). Diadromous fishes may have substantial osmotic, bioenergetic and predation-exposure costs in moving between two environments, but they benefit from generally reduced predation during early life stages in rivers and migration may provide access (in non-tropical marine waters) to the greater trophic resources of the marine environment (Gross 1987). Due to the diadromous life-cycle displayed by *L. fluviatilis*, the most pervasive factors contributing to their decline are river regulation and obstruction (Tuunainen *et al.* 1980; Renaud 1997; Close e*t al.* 2002).

The majority of lowland rivers in Europe have been modified to some extent (Cowx & Welcomme 1998) and migration barriers are now recognised as one of the key threats to freshwater fishes worldwide (Baras & Lucas 2001), particularly for the recovery of affected populations (Albanese *et al.* 2009). Any modification to hydrological regimes may, temporarily or permanently, reduce or eliminate the connectivity between rivers and tributaries with inherent implications for both resident and migratory species (Nunn *et al.* 2010). Negative, cumulative, effects of multiple partial barriers on upstream migration have been observed for Atlantic salmon (*Salmo salar*, Gowans *et al.* 2003) and for Pacific lamprey (*Entosphenus tridentatus*, Moser *et al.* 2002) at large hydroelectric dams. Impacts due to barriers seem to vary among closely related species, which is consistent with general observations that species' responses to habitat fragmentation are often diverse and challenging to predict (Debinski & Holt 2000; McLaughlin *et al.* 2006). When the distribution of key habitats in the catchment is overlaid relative to the

distribution and nature of such barriers, a system with selective permeability to and from habitat fragments is created (Lucas *et al.* 2009). Populations that are physically and genetically isolated may suffer from decreasing population sizes and inbreeding, which may increase the risk of extinction (Brook *et al.* 2002; Morita *et al.* 2009).

Nunn *et al.* (2008) found evidence of variable year class strength of *Lampetra* larvae in the Ouse catchment (north-east England), especially upstream of barriers, and have suggested that this was a reflection of limited access by adult lampreys, linked to flows in some years. The availability of high flows for enabling passage is likely to have a strong effect on the access to spawning habitat fragments, and hence on the extent of the nursery area downstream from which the weakly swimming larvae are recruited (Lucas *et al.* 2009). McLaughlin *et al.* (2006) found that barriers did not differentially affect freshwater fish species (mostly non-diadromous and non-lamprey species) from certain genera or families, nor did they affect certain body forms, meaning taxonomic affiliation and swimming morphology are not useful in predicting sensitivity to barriers. McLaughlin *et al.* (2006) found that the majority of taxa that they examined did not exhibit evidence of being affected by low-head barriers, however a potentially meaningful proportion did. *Petromyzon marinus* in Portugal were delayed by block weirs of less than 1m in height and these fish expended large amounts of energy trying to pass these obstacles (Almeida *et al.* 2002; Almeida *et al.* 2005).

The behaviour of adult lampreys seeking to pass low-head barriers usually involves the use of the sucker for attachment to the surface of the barrier, interspersed with burst swimming, usually in lower flow areas and often around the edges of fully or partially inundated barriers (Beamish 1974; Hardisty 1986b; Quintella *et al.* 2004; Reinhardt *et al.* 2009; Kemp *et al.* 2010). However, studies have shown that several species of lamprey

(e.g. *E. tridentatus* and *Geotria australis*) exhibit highly efficient climbing behaviour on steeply inclined surfaces, which aids passage at low-head barriers (Jellyman *et al.* 2002; Moser *et al.* 2002; Reinhardt *et al.* 2008). Both eels (*Anguilla* spp.), and lampreys swim by lateral undulatory movement, a style which is thought to be relatively inefficient compared to other biomechanical modes of swimming (Sigvardt 1989). This poor swimming ability provides a challenge when they encounter man-made structures and reservoirs during the upstream migration (Dauble *et al.* 2006). However, recent studies have shown that in European eels (*Anguilla anguilla*), this form of undulatory locomotion is actually highly efficient (in terms of energy per km travelled) over long distances (van Ginneken *et al.* 2005; Burgerhout *et al.* 2013) which would prove advantageous to anadromous lampreys that also undertake energetically costly, long distance migrations.

One way of enabling the upstream passage of lampreys is to employ fishways, yet for many species most of these are not very effective (Lucas & Baras 2001; Moser *et al.* 2002). It has been shown that lamprey migration is often delayed for over 5 days as they negotiate fishways designed to facilitate salmonid passage (Moser *et al.* 2003). A recent study using Passive Integrated Transponder (PIT) telemetry has shown that pool-weir and Denil style fishways (a series of symmetrical close-spaced baffles or vanes, a type of vertical slot fishway) had a respective attraction for lampreys of 43% and 92%, however lampreys failed to pass despite re-entering on up to 12 separate days, and could be delayed for up to 150 days (Foulds & Lucas 2013).

Lucas *et al.* (2009) found that lampreys would favour barrier passage during periods of high flow and that fishways were unimportant in achieving passage at these times. Every obstruction, even if fitted with effective fish passage facilities, will create at least some delay in both upstream and downstream migration (Larinier 2008), and sometimes delay

of upstream migrants can occur simply from difficulty in finding a fishway (Schilt 2007). Once the fishway has been successfully located the potential for a further type of delay called 'fallback' can also occur and can be particularly harmful. This is when a fish leaves the fishway to be drawn back downstream through some other passage route such as a turbine intake, or simply moves back downstream at a greater frequency than would occur in unimpounded reaches (Reischel & Bjornn 2003). Lamprey fitness, therefore, may be reduced by causing excessive use of energetic reserves, and/or direct mortality by predators at the base of obstructions (Moser & Mesa 2009).

*1.3.2.1 Delay in Migration*

Diadromous fish must undergo profound physiological changes to be able to make such a transition from a marine to freshwater environment, or vice versa (Folmar & Dickhoff 1980; Youson 1980), and being either delayed or accelerated due to the presence of anthropogenic barriers could reduce their survival in the new environment. Therefore, the delay of downstream or upstream migrants should be reduced as much as possible (Castro-Santos & Haro 2003). In general, impounded rivers pass fish downstream more slowly than do free-flowing rivers and can cause individuals to arrive at the marine or estuarine environment at a later time with lower energy reserves than they would have in a free-flowing river (Schilt 2007).

The tailrace environment at dams can also be the site of delay for downstream migrants due to disorientation and stress from passage, water circulation patterns that may hold migrants, or an increase in the level and duration of predation caused by the concentration of downstream migrants into one area, allowing predators to congregate (Čada 2001). It has been found that fish can also prove reluctant to move downstream over a weir and may instead, return upstream (O'Connor *et al.* 2006). This has been

observed in brown trout smolts (*Salmo trutta*) and Atlantic salmon (Hansen *et al.* 1984; Aarestrup & Koed 2003), and a similar response has also been observed in downstream migrating European eels (*Anguilla anguilla*, Behrmann-Godel & Eckmann 2003). Although these obstructions may not affect a population physically, this observed avoidance behaviour can also delay downstream migration.

*1.3.2.2 Access to spawning areas*

With regard to upstream migration, the appropriately timed arrival of potential mates and conditions for reproduction are vitally important and must coincide with the physiological timing of lamprey sexual maturation (Schilt 2007; Lucas *et al.* 2009). Delay during this migration, however, can result in a reduced chance of reaching appropriate spawning areas within a constrained timescale. In some rivers, particularly those with large barriers and/or relatively stable discharge, access to spawning grounds may depend partly upon synchronisation of the lamprey spawning migration with elevated river levels (Nunn *et al.* 2008).

Where spawning areas are limited downstream of an obstruction, reproduction, and the subsequent recruitment of lampreys within that location, may be very limited (Lucas & Frear 1997). Large spawning aggregations in discrete localities are extremely susceptible to interference, habitat degradation, or environmental perturbations (Jang & Lucas 2005). For promiscuous, non-territorial spawners such as *L. fluviatilis,* it is possible that tiny fragments of spawning habitat are sufficient for effective spawning and do not represent a significant bottleneck (Lucas *et al.* 2009). However, if only a small number of sites are used intensively for spawning by adult lamprey, the likelihood of severe damage by catastrophic events such as pollution, river engineering, or local exploitation (Masters *et al.* 2006) is much greater. There is, therefore, a need to facilitate upstream passage at

potential physical obstructions to improve access of migrating lamprey to under-utilised spawning and nursery areas (Nunn *et al.*, 2008).

### 1.3.3 Hydropower

As well as delays caused to both upstream and downstream migration by barriers, the addition of a hydropower turbine can lead to additional potential risks of entrainment (being drawn into the turbine) or impingement (a collision) with debris screens. Passage through turbines may cause a range of damage to fish, depending on the type and size of turbine, species, size, and behaviour of fish, velocity of water, speed and magnitude of pressure fluctuations within the water in close proximity to turbine blades, roughness of materials, and the force and direction of contact with blades or other parts of the turbine (Coutant & Whitney 2000).

Formerly, freshwater hydropower impacts on fish were principally associated with large dams, but currently, commitment to increasing sustainable energy production has triggered the mounting number of low-head (<10 m) hydropower schemes. For example, following construction of impassable hydroelectric dams in Finland, populations of European river lamprey have declined (Tuunainen *et al.* 1980; Ojutkangas *et al.* 1995). Low-head hydropower is developing rapidly within the UK in response to business opportunities, assisted by EU and UK policies of encouraging renewable energy and reducing reliance on fossil fuels. Despite the fact that absolute barriers are not formed, upstream movement may be restricted to only a limited range of environmental conditions (Lucas & Frear 1997; O'Connor *et al.* 2006). In addition, hydropower facilities sited at dams and weirs may increase mortality of migrating fishes, especially in a downstream direction (Čada 2001). Considering that juvenile (i.e.

ammocoetes and transformers) lamprey commonly exhibit downstream drift or, in the case of transformers active emigration, they may be at risk from entrainment.

For these reasons, Archimedes' screw turbines have been introduced as a 'fish friendly' alternative to conventional propeller-type turbines. The relatively slow rotational speeds, limited shear forces and small pressure changes within these turbines mean that, compared to most other types of turbines available, they should have a relatively low impact on fish (Spah 2001; O'Keefe & Turnpenny 2005). However, although Archimedes' screw turbines are becoming increasingly popular, as with other mitigation efforts, studies have been heavily biased toward salmonid passage. The vastly different body type of lampreys, particularly with regard to the smaller larval and transformer stages, which are the most likely to pass downstream through turbines, suggests that they will be affected differently in passage through a turbine. Considering that these structures are being constructed at numerous points through river systems (some containing Special Areas of Conservation designated because of the lamprey populations present) and that it is likely that some proportion of the lamprey populations should pass through these structures at some point, it seems almost essential that further study is directed towards assessing the impact on lampreys.

## 1.4 Lamprey Species Pairs

A trend in the evolution of Petromyzontiformes is the occurrence in most genera of 'paired species' (Zanandrea 1959). The larvae of paired species are morphologically similar but the adults adopt different life-history strategies, either becoming a freshwater resident non-parasitic type or an anadromous parasitic type. As more than one non-parasitic species may be derived from a given parasitic anadromous species, the term

'satellite species' can also be used in place of 'paired species' (Vladykov & Kott 1979). Paired or satellite species occur in seven of the ten lamprey genera, and it is generally accepted that lamprey paired species are closely related, the non-parasitic freshwater species having evolved from a similar form to that of the extant parasitic anadromous lamprey (Zanandrea 1959; Hardisty 1986a; Schreiber & Engelhorn 1998; Youson & Sower 2001; Gill *et al.* 2003; Renaud *et al.* 2009; Docker *et al.* 2012). There has, however, been much controversy about the taxonomic status of paired lamprey species (Renaud *et al.* 2009).

The wholly–freshwater, non-parasitic *L. planeri* and the anadromous ectoparasite *L. fluviatilis* are considered to be 'paired species' whose larvae cannot usually be differentiated externally (Potter & Osborne 1975). Consequently, this can lead to problems when trying to assess species' abundance or determine management stocks. A shift from an anadromous to a freshwater life history is not an unusual occurrence. Due to the nature of anadromy, breeding will occur in freshwater providing the opportunity to colonise this environment. The reason for availing of this might be due to the cost of migration exceeding the value of marine food resources (Bell & Andrews 1997).

In diadromous fish, multiple independent divergences of freshwater populations through repeated independent evolution of the same reproductive isolating mechanism seems to be a common trend (Schluter & Nagel 1995). Genetic divergence between populations will occur when reproductive isolating mechanisms, preventing gene flow between them, are in place. Speciation was originally thought to require geographic isolation of populations in order to prevent gene flow. This is known as allopatric speciation (Mayr 1942) and requires the physical isolation of populations, which then leads to their differentiation by the process of genetic drift and local adaptation.

However, it has now become evident that geographical barriers are not necessarily required for the process of speciation and that other non-allopatric processes (i.e. ecological) such as natural selection are more important than previously thought (Mayr 1942; Schluter 1996; Bush 2001; Schluter *et al.* 2001).

It has been suggested that this loss of anadromy might act as an initiator for radiation and speciation (Bell & Andrews 1997; Lee & Bell 1999). A critical early stage in speciation is the evolution of genetic differences between populations, and populations that colonise novel environments can evolve extremely rapidly (Carroll *et al.* 1997; Losos *et al.* 1997; Reznick *et al.* 1997). This is due to the fact that these populations may originate from a few founders leading to the increased possibility they could experience large changes in allele frequency from genetic drift (Carson & Templeton 2003). This could cause substantial morphological change and reproductive isolation resulting in an 'adaptive radiation' of a morphologically novel and rapidly diverging population (Coyne 1992). Glaciation, for example, may have promoted evolution of non-parasitic lamprey species by either blocking migratory routes and preventing anadromy or upon deglaciation making available new habitat and food resources that are inaccessible through freshwater, but easily reached by anadromous fish (Bell and Andrews 1997). It has also been suggested that changes in the environment, in particular the formation of new barriers to migration or the reduced availability of host fishes (e.g. through over-exploitation), might promote a complete abandonment of adult feeding (Hardisty 1986a). In addition, habitat fragmentation reduces population sizes and consequently genetic diversity due to a faster rate of inbreeding and/or a greater impact of genetic drift than that observed in larger populations (Frankham *et al.* 2002).

It is possible that multiple occurrences of *L. planeri* may result from independent divergences from *L. fluviatilis*, meaning that resident and migratory populations from the same river would be genetically more related to each other than populations of the same species in differing catchments. Improved understanding of the systematics and population genetic structure could aid lamprey conservation (Rodriguez-Munoz *et al.* 2004) through the better identification of management stocks. Furthermore, increasing numbers of barriers may cause increased isolation of some populations and this may be determined through population genetic structure. To date, no studies have used microsatellite markers to examine the *L. fluviatilis* and *L. planeri* species-pair puzzle. Microsatellites are repeating units of DNA that occur frequently, and randomly, in all eukaryotic nuclear DNA genomes (Tautz & Renz 1984; Gupta *et al.* 1994). Microsatellites are neutral markers (i.e. they are unaffected by natural selection) and are, therefore, useful for providing information on genetic variation caused by alternative evolutionary processes such as gene flow and drift. Through analysis of molecular markers, insights are provided into population divergence and dispersal at local to catchment scales. This allows inferences to be drawn about population connectivity and evolutionary viability, and has important applications in conservation management and the identification of ESUs (Latta 2008).

## 1.5 Lamprey Pheromones

Pheromones are defined as 'an odour or mixture of odours released by the sender that evokes in the receiver(s) adaptive, specific, and species-typical response(s), the expression of which need not require prior learning or previous experience' (Sorensen & Stacey 2004). Pheromones may be utilised by many different organisms as chemical cues for various types of behaviour, such as, predator avoidance (Friesen & Chivers 2006),

migration (Nordeng 1977), shoaling (Mann *et al.* 2003), and reproduction (Kobayashi *et al.* 2002). In migratory species, mechanisms of orientation are vital for allowing an individual to complete its life cycle. In an aquatic environment, communication via water soluble chemicals is ideal as this enables the transfer of information over large distances (Burnard *et al.* 2008). This ability to use pheromones for low-cost, long-distance communication can have significant evolutionary implications as it allows animals at low densities to convey messages regarding the location of potential mates (Wyatt 1956) and suitable habitats in which they may reproduce.

Due to the parasitic feeding strategy of sea lamprey *Petromyzon marinus* and the European river lamprey *Lampetra fluviatilis,* finding a spawning site with potential mates may be problematic as there is a significant chance they will become widely dispersed through transport by the diverse hosts they parasitise. Waldman *et al.* (2008) suggest that the presence of parasitism has coevolved with an alternative reproductive strategy for anadromous fish species, a strategy that, instead of homing, allows an individual to chemically recognise the presence of conspecifics and potentially suitable freshwater habitat for spawning. Olfactory cues are involved in both strategies, the use of which allows an individual to reinforce the reliability of spawning habitat prior to an energetically costly upriver migration.

The role of olfaction in fish migration has been examined in great depth (Stabell 1992). Salmon use olfactory cues to locate their home stream (*Oncorhynchus nerka*, Hasler & Wisby 1951), as do shad (*Alosa sapidissima*, Dodson & Leggett 1974). In some migratory fish species this is based on within-individual 'memory' as predicted by the imprinting hypothesis (Hasler & Wisby 1951) which claims that homing depends on recognition of specific stream odours that were learned and imprinted during the juvenile stage. On the

other hand, the pheromone hypothesis, in the context of migratory salmonids (Nordeng 1971, 1977), maintains that population specific odours emanating from both juvenile conspecifics residing in freshwater, and those migrating to sea, guide the homing adults. These theories, however, are not necessarily mutually exclusive and some combination of the two may influence lamprey upstream migration.

It was Teeter (1980) who originally suggested a correlation between ammocoete abundance and stream selection by landlocked adult sea lamprey (*P. marinus*) in the Laurentian Great Lakes and attributed this to some form of pheromonal odour released by larvae. There was some speculation that natal stream fidelity may be the underlying cause, as is the case in some other anadromous species such as Pacific salmon (*Oncorhynchus* spp.) (Dittman & Quinn 1996), Atlantic salmon (*Salmo salar*) (Marschall *et al.* 1998) and many other salmonids (McDowall 2001). However, Bergstedt and Seelye (1995) concluded that *P. marinus* exhibited no obvious homing behaviour and that spawning streams seemed to be selected through innate attraction to other sensory cues. This was ascertained by tagging 555 metamorphosing larval *P. marinus* and surveying numerous streams to see, as adults, how they would distribute themselves. It was found that out of the ~10% of marked animals that were recaptured, none returned to their natal streams.

Waldman *et al.* (2008) used mitochondrial DNA collected from 11 North American east coast rivers to examine genetic evidence for natal homing in *P. marinus*. No significant differences in haplotype frequencies were found between locations, and combined with evidence from other studies using microsatellite DNA (Bryan *et al.* 2005), it was concluded that *P. marinus* do not return to their natal rivers. This absence of homing has

resulted in *P. marinus* exhibiting regional panmixia within a large geographic area, which consequently raises the question of how *P. marinus* locates suitable spawning rivers (Beamish 1980). The choice of river for spawning is critical for anadromous lampreys such as *P. marinus,* as they expend a considerable amount of energy for gamete production and die shortly after spawning, leaving a finite amount of energy available for the upriver migratory phase (Beamish 1979). *Petromyzon marinus* seemingly use a novel strategy not to home as such, but to chemically identify habitats suitable for spawning based on recognition of the presence of conspecifics (Bergstedt & Seelye 1995; Sorensen *et al.* 2003; Waldman *et al.* 2008).

Whilst trying to eradicate invasive sea lampreys from the Upper Laurentian Great Lakes, Moore and Schleen (1980) found that removing larval lampreys using lampricide (trifluroromethyl-4-nitrophenol (TFM)) significantly affected the subsequent recruitment (by about half) within that system. In the year following larval removal, adult *P. marinus* that would normally have entered the treated system were instead located in surrounding streams whose level of relative attractiveness increased simply for not having being treated. It was subsequently revealed that, as a larva, *P. marinus* produces a multi-component steroidal pheromone comprising of a mixture of four sulphated steroids; petromyzonol sulphate (PS), petromyzonamine disulphate (PADS), petromyzosterol disulphate (PSDS) and allocholic acid (ACA) that attract adults of both sexes in the early migratory phase (Li *et al.* 1995; Li & Sorensen 1997; Bjerselius *et al.* 2000; Polkinghorne *et al.* 2001; Vrieze & Sorensen 2001; Sorensen *et al.* 2005).

Behavioural experiments, conducted in both the laboratory and the field, have provided evidence that adult *P. marinus* do, in fact, select spawning rivers based on the odour of larvae that they contain and that bile acids released by the larvae are part of this

pheromonal odour (Bjerselius *et al.* 2000). Migratory adults in a laboratory maze have been shown to exhibit enhanced swimming activity in the presence of a 0.1 nM concentration of the two unique bile acids released by larvae (Bjerselius *et al.* 2000).

Wagner *et al.* (2006) subsequently conducted a field test during which larval holding water was added to river water. It was found that migrating *P. marinus* showed a preference for the branch of the river in which the larval holding water had been added. This natural larval odour has been found to be attractive to adult *P. marinus* of both sexes in large mazes in the laboratory and mazes placed in a stream (Sorensen *et al.* 2003). It seems that *P. marinus* have evolved such a strategy because the presence of larval lampreys signifies the presence of nursery habitats and, by default, spawning habitat as well (Fine *et al.* 2004). Immature (non-migratory) lamprey do not seem to share this attraction and adult females, which are fully mature and ovulating, also seem not to exhibit this preference, the latter responding instead to odours released by spermiating males (Bjerselius *et al.* 2000; Vrieze & Sorensen 2001). *Petromyzon marinus* is now also known to demonstrate pheromone mediated behaviour in the form of a reproductive cue (3-keto petromyzonol sulphate (3kPZS) and 3-keto allocholic acid (3kACA)) released by sexually mature males to attract ovulating females (Li *et al.* 2003; Yun *et al.* 2003b). Considering that there is only a one enzyme difference between PS and ACA produced by larval lampreys, and 3kPZS and 3kACA produced by spermiating males, it seems likely that the pathway of biosynthesis for these pheromones is the same (Yun *et al.* 2003b).

Generally, it seems that closely related species of fish have similar pheromone compounds and distantly related species have dissimilar ones (Burnard *et al.* 2008). A slight variation in mixture, however, has been shown to be enough to avoid

hybridisation between some closely related species of teleost fish (Sorensen & Scott 1994). Other closely related fish species (e.g. cyprinids), however, have been shown to produce similar sex pheromones (Irvine & Sorensen 1993) that can elicit a mating response in heterospecific individuals. This has also been well illustrated with hybridisation between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (Essington & Sorensen 1996). It would be expected that sympatric species would also possess different sex pheromones (Burnard *et al.* 2008), so as to avoid inter-species breeding but again this is not always the case as male Atlantic salmon (*S. salar*) and brown trout (*S. trutta*) have shown similar hormonal responses to ovarian fluid and the urine of both conspecific and heterospecific females (Olsen *et al.* 2000).

Biochemical and behavioural evidence has suggested that the migratory pheromone of *P. marinus* may not be completely species-specific (Fine *et al.* 2004). The larval holding waters of American brook lampreys (*Lethenteron appendix*), northern brook lampreys (*Ichthyomyzon fossor*), and *P. marinus* were, on separate occasions, metered into one side of a two-choice maze with non-lamprey river water. This resulted in adult *P. marinus* spending *c.* 65% of their time in the odour of each of the three species of larvae (rather than the control), showing the possibility of overlap between cues released by discrete lamprey species (Fine 2001; Fine *et al.* 2004). Further behavioural studies using adult *P. marinus* in their migratory phase found that they were attracted to the odours of both heterospecific and conspecific larvae and that their holding waters contained similar amounts of PS (Fine *et al.* 2004). Biologically significant concentrations of odour released from two heterospecific species of larval lamprey (*I. fossor* and *L. appendix*) were strongly attractive to migratory *P. marinus*, and adult silver lamprey (*Ichthyomyzon unicuspis*) were attracted to the odour of larval *P. marinus* (Fine *et al.* 2004). This suggests the

release of PS is not a specialised trait but rather a common one to many, and perhaps all, members of the family Petromyzontidae.

In fish, bile acid production has been found to vary between families, but not at any lower taxonomic level (Hoshita 1985). This raises the question of how specific a pheromone, which is partially comprised of bile acids, might be and how this may have evolved (Fine *et al.* 2004). It is possible that this overlap reflects a lack of specialisation in pheromone production, or alternatively, an inability of the adult olfactory system to discern cues released by other petromyzontid species (Sorensen & Vrieze 2003). Fine (2001) maintains that it is reasonable for petromyzontid lampreys to employ a common ancestral migratory cue as, historically, little overlap existed between species. Anthropogenic effects, however, have altered the distribution of many lamprey species which has led to a situation of co-habitation which may not have previously existed. Conceivably, heterospecific individuals could therefore be utilising larval cues (such as metabolic by-products) in a similar fashion to similar to conspecific individuals in which a migratory reaction is illicited. The potential utility of larval pheromones as an aid to guiding adults to suitable spawning streams (Burnard *et al.* 2008), or re-establishing a population (Gaudron & Lucas 2006) would also be extremely valuable from a conservation perspective.

## 1.6 Thesis Aims

The principal focus of this thesis is to investigate the evolutionary and behavioural ecology of the European lamprey species pair *Lampetra fluviatilis* and *Lampetra planeri* in relation to conservation concerns. This will be achieved by employing a range of approaches, including, ecological, behavioural, and population genetic based research, to

bridge gaps in knowledge surrounding this species pair. This thesis aims to offer a holistic approach to the management and conservation of lampreys in relation to anthropogenic impacts. This will be achieved within this thesis as follows:

- Chapter Two will assess the potential impact that Archimedes screw hydropower turbines could have on downstream moving lamprey populations and, within this context, examine patterns of migration and drift of larval and transformer stage lampreys.

- Chapter Three examines the genetic diversity, and levels of differentiation between several *L. fluviatilis* and *L. planeri* populations from the British Isles and northern Europe using mitochondrial DNA markers. This should provide information about the timing of divergence between the two species and offer insight in to the demographic history of this species pair.

- Chapter Four examines the development and utilisation of polymorphic microsatellite loci to test whether populations of *L. planeri* are genetically closer to populations of *L. fluviatilis* within the same river than to allopatric populations of *L. planeri*. This will be carried out on varying spatial scales with samples from both species being compared. Combining this information with previous information gathered from mtDNA analysis, the hypothesis that the post-glacial expansion of anadromous *L fluviatilis* populations into northern Europe prompted the independent establishment of multiple populations of *L. planeri* will be tested. Microsatellite loci will also be used to estimate both contemporary and long-term gene flow between species. The assessment of biodiversity within populations is essential in identifying areas of priority for conservation and

management. Factors that may affect levels of biodiversity, such as barriers to migration, will also be examined to enhance our understanding of the effects of fragmentation within riverine ecosystems and consequently support more effective management strategies for lamprey in the future.

- Chapter Five will synthesise and discuss the findings of Chapters Two to Four and their overall implications for lamprey conservation. This chapter will also outline future recommendations and directions for lamprey conservation and management.

“Earth provides enough to satisfy every man's needs, but not every man's greed.” — *Mahatma Gandhi*

“Men may dam it and say that they have made a lake, but it will still be a river. It will keep its nature and bide its time, like a caged animal alert for the slightest opening. In time, it will have its way; the dam, like the ancient cliffs, will be carried away piecemeal in the currents.” — *Wendell Berry*

# Chapter 2 Potential impacts of small-scale hydroelectric power generation on downstream moving lampreys

## 2.1 Introduction

For centuries civilisations have been harnessing the power of water (Poff & Hart 2002). The basic water wheels that were once used to power mills for grinding wheat into flour have given way to highly-efficient turbines that are considered one of the most important renewable energy sources worldwide (Bratrich *et al.* 2004) accounting for over 19% of the world's electricity (Paish 2002). On a constantly developing planet, with an ever increasing human population, our energy needs are constantly growing. To meet this demand, new methods for harnessing energy are continually being explored, however, renewable and carbon neutral energy are particularly needed to help mitigate the effects of climate change. In this respect the main advantage of hydropower, along with the fact that it provides a steady and secure source of electricity compared to the intermittency of other renewable energy technologies such as solar photo-voltaic and wind power, is that it is 'clean' i.e. does not produce any waste products that may be harmful to the environment.

In January 2008, the European Commission published a proposal to fight climate change and promote renewable energy. A Directive (the Renewable Energy Directive) was proposed that provides the legislative base to implement a binding 20% renewable energy share (in energy consumption) by 2020 (i.e. 20% of energy produced must come from renewables by 2020). Although wind power is likely to be the principal energy harnessed in this endeavour, hydropower also has a significant role to play. In Europe, most of the large-scale hydropower opportunities have already been exploited or are

otherwise considered environmentally unacceptable, however, strong potential remains for small-scale hydropower, and the number of such schemes is increasing rapidly (Paish 2002; Kosnik 2010). The International Energy Agency (IEA) predicts that the hydropower output worldwide will increase from 2809 TWh (tera watt hours) in 2004 to 4749 TWh by 2030. In England and Wales alone it is estimated that the number of freshwater hydropower facilities will rise from 400 to 1200 by 2020 (Environment Agency 2010).

Hydropower is often presented as an environmentally benign renewable energy source and was considered to be the most reliable and cost effective source in the UK for almost 10 years between 1997 and 2006 (San Bruno *et al.* 2008), being overtaken by wind power in 2007 (DECC 2010). Large hydropower schemes (i.e. generally with a capacity > 10 MW (megawatts)) typically use dams to store a reservoir of water. In contrast, small-scale (i.e. with a capacity of < 10 MW) run-of-the river schemes (i.e. little or no water storage) divert a proportion of the river flow through turbines before returning the water downstream. Run-of-the river schemes, however, generally still require an impounding structure of some description. A rich historic resource of weirs and old mills in the UK provides an ideal opportunity for the re-development of existing sites with the retro-fitting of hydropower schemes to these structures. In low head (< 10m) run- of –the –river schemes the water is often diverted via an existing mill leat and channelled through a screened turbine before release downstream. If a turbine is placed on an existing weir structure, the water is usually returned to an existing weir pool, which avoids any depletion within a water course that can occur with a diversion of water to a turbine offset from the main river.

Due to small-scale hydro schemes being both reliable and one of the most economic methods of generating electricity, together with having the capacity to respond immediately to fluctuations in demand, they are considered by some to be the backbone of electricity production in many EU countries (San Bruno 2008). In larger schemes, however, a pre-determined volume of water produces a more reliable power supply (Jansson 2002) compared to small schemes that are dependent on ambient river flows. In the UK, financial incentives, such as the feed-in tariff introduced in April 2010, will guarantee a high fixed price for hydropower generated energy for up to 20 years. The latter, coupled with the Renewable Energy Directive introduced in 2009, is further likely to encourage the emergence of small-scale hydropower facilities. However, although hydropower is portrayed as having no negative impacts on the environment in the context of chemical by-products, the impacts on fisheries and other riverine biota may be significant.

In England and Wales, the Environment Agency supports the principle of expanding renewable energy through low-head hydropower and has identified nearly 26,000 potential sites which, if all were developed, could provide 1% of the UK's electricity needs (Environment Agency 2010). In Scotland, a further 36,000 sites have been earmarked for potential development (Forrest *et al.* 2008). However, there is also a requirement to ensure that such developments do not compromise ecological integrity and biodiversity. In December 2000, the Water Framework Directive (WFD) was established. This is a legal framework for the protection, improvement and sustainable use of rivers, lakes, estuaries, coasts and groundwater across Europe. The WFD signifies a commitment from countries within the EU to achieve or maintain a 'good ecological

status' by 2015. An integral part of achieving this goal is the freedom of movement of fish, both in an upstream or downstream direction.

Although hydropower installations are likely to have a wide variety of effects on both the physical and biological constituents within a fluvial system (Čada & Hunsaker 1990; Robson *et al.* 2011), those at greatest risk of impact are fishes (Lucas & Baras 2001). In particular, species that rely on regular migrations on a seasonal, or life-cycle basis (Baras & Lucas 2001), will require the longitudinal connectivity of rivers to be upheld. As previously outlined (Chapter 1, Section 1.3.2), potential risks include: delays to migration, disorientation, increased exposure to predation, as well as direct mortality and injury (Office for Technology Assessment 1995; Turnpenny *et al.* 1998; Coutant & Whitney 2000; Čada 2001; O'Keefe & Turnpenny 2005). Thus considerable efforts have been made to identify species at risk and to minimise impacts of hydroelectric facilities on fish migrating downstream and upstream. Key elements of these processes include appropriate screening, proper siting of facilities relative to flow patterns, provision of efficient upstream and downstream fish passage routes, and minimising access to dead ends (Office for Technology Assessment 1995; Turnpenny *et al.* 1998; Coutant & Whitney 2000).

Anthropogenic barriers such as barrages, dams, and weirs can lead to the fragmentation and isolation of fish populations (Baras & Lucas 2001; Morita & Yamamoto 2002; McLaughlin *et al.* 2006) and the lack of availability of one or more habitats, or even poor connectivity between these habitats, is likely to act as a bottleneck and lead to population decline (Wilcox & Murphy 1985; Law & Dickman 1998). Effects of barriers,

turbines and water diversion on mortality and access to habitat are a conservation concern for anadromous species, both for existing and recovering populations (Lucas & Baras 2001).

Lampreys are one group of fishes that are sensitive to the impacts of river barriers and habitat modification, including hydropower generation (Moser *et al.* 2002; Lucas *et al.* 2007; Lucas *et al.* 2009). Anadromous lamprey species, in particular, require free migration to the sea at the macrophthalmia ('transformer') stage and back to spawning areas in rivers as mature adults. Over half of all lamprey species are considered to be endangered, vulnerable, or extinct in at least a portion of their range (Renaud 1997) and marked declines in the abundance of anadromous lampreys have been attributed to human activities (McDowall 1992; Renaud 1997; Kelly & King 2001; Raat 2001; Close *et al.* 2002; Masters *et al.* 2006; Mateus *et al.* 2012). In Europe, sea lamprey *Petromyzon marinus*, European river lamprey *Lampetra fluviatilis* and European brook lamprey *Lampetra planeri* are afforded protection through the European Commission (EC) Habitats and Species Directive, which requires Special Areas of Conservation (SACs) to be identified and maintained in good condition for these species (EC 1992). Regulatory control is applied to factors within or outside SACs that are likely to damage the condition of interest features within SACs. For lampreys, these factors include poor upstream access at barriers (Lucas *et al.* 2009) but also potential impacts to emigrating lamprey and drifting ammocoetes (larvae) passing through hydroelectric turbines (Lucas *et al.* 2007). Impacts on downstream-moving mature adults are of somewhat lower concern as migration is principally directed upstream and all lamprey die soon after spawning.

Until recently, the underlying research and mitigation methods concerning anthropogenic impacts on migrating fishes have been strongly biased towards the needs of anadromous salmonids and, to a lesser degree, a few other taxa (Lucas & Baras 2001). For example, the mesh size of angled bypass screens to deflect downstream-migrating fish from water intakes in the UK is commonly 10-12 mm, a size which satisfactorily prevents Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* smolts from gaining entry (Turnpenny *et al.* 2000) but will not exclude juvenile lamprey. Increasingly, regulatory bodies have given greater attention to other taxa and smaller life stages, including young lamprey, which may be susceptible to mortality during turbine passage (Dadswell & Rulifson 1994). Larval and juvenile lampreys can easily be entrained through water intakes, and this has resulted in increased use of finer mesh or narrow bar-space screens (e.g. 3 mm spacing) to prevent access (O'Keefe & Turnpenny 2005). In high flows, weakly swimming species and life stages can be impinged on screens, causing high mortality (O'Keefe & Turnpenny 2005) and this is a significant problem for juvenile Pacific lamprey *Entosphenus tridentatus* (Moursund *et al.* 2003; Dauble *et al.* 2006; Sutphin & Hueth 2010) and probably also for other lamprey species. For low-head (< 10m), small-scale, hydropower schemes fine-mesh screens are likely to hamper operation and dramatically reduce their efficiency.

Passage through turbines may cause a range of damage to fish, depending on the type and size of turbine, species, size and behaviour of fish, velocity of water, speed and magnitude of pressure fluctuations, roughness of materials, and the force and direction of contact with blades or other parts of the turbine (Office for Technology Assessment 1995; Coutant & Whitney 2000; Turnpenny *et al.* 2000; Cooke *et al.* 2011). In general, the greatest impacts of traditional propeller (e.g. Kaplan-type) turbines are observed on large anguilliforms (eel-shaped), moving downstream (e.g. adult European eels *Anguilla*

*anguilla*), and on fishes that lose scales easily or have a 'delicate' anatomy (e.g. clupeids such as shad *Alosa alosa*). It is well documented that lampreys are susceptible to impingement and entrainment at abstraction sites due to their elongated shape, their poor swimming capabilities and lack of avoidance to accelerating flows (Russon & Kemp 2011; Rose & Mesa 2012). Both entrainment and impingement can lead to fatigue, damage and mortality of lampreys (Moursund *et al.* 2003; Rose & Mesa 2012). On this basis, it might be expected that adult lampreys (*L. fluviatilis* or *L. planeri*) could be affected if they were to move down through turbines. Emigrating transformers and drifting ammocoetes entering turbine chambers would be expected to be less susceptible to major damage by virtue of their small size and body characteristics (O'Keefe & Turnpenny 2005). Moursund *et al.* (2003) found no evidence of health impacts on *E. tridentatus* transformers as a result of simulated turbine shear stress and pressure fluctuations, similarly a field study at a hydropower station on the River Tay, Scotland, found no evidence of significant impact on *Lampetra* spp. larvae (Lucas *et al.* 2007).

Rapid escalation in low-head, run-of-the-river hydroelectric development in the UK and elsewhere in Europe has occurred in concurrence with the introduction of the Archimedes screw turbine (Spah 2001; Kibel 2007). These systems are relatively robust, low-maintenance, hydroelectric screw turbines that can operate over a range of flows. The force of water rotates the screw's blade and the mechanical power is converted to electrical power. These screw turbines are regarded as more fish friendly than conventional designs, because of the relatively slow rotational speeds, limited shear forces and small pressure changes compared to conventional turbines (Spah 2001). Low rates of injury have been recorded for several non-lamprey species experimentally passed through screw turbines in some studies (Spah 2001; Kibel 2007) but not others (Schmalz 2010). Injuries to fish passing through Archimedes screw turbines, especially

to small, slender fish such as young lampreys are most likely to result from pinching between the screw blade and the trough. The aim of this study was to assess the potential for impacts of Archimedes screw turbines on downstream-moving juvenile and larval lamprey.

## 2.2 Study Area

The (Yorkshire) River Derwent (mean discharge of *c.* 15 $m^3 s^{-1}$) is a tributary of the River Ouse (Figure 2.1) that joins the River Trent to form the River Humber (mean discharge of 250 $m^3 s^{-1}$) in north-east England (Law *et al.* 1997). In its headwaters, the Derwent is a shallow, fast flowing, upland river. In the lower 55 km, it is a slower, deep, lowland river, with a very low gradient. Much of the drop in the lower river occurs at a series of weirs, where several small-scale hydropower plants exist or are planned. The lower Derwent does not presently have a significant migratory salmonid population and is characterised by a lowland river fish community (Whitton & Lucas 1997). Freshwater spawning habitat (i.e. gravel substrate with fast moving well oxygenated flowing water) and larval habitats (silt/sand substrate with slow moving water) for lampreys are present in Ouse tributaries, including the Derwent, which provides suitable conditions for a substantial river lamprey population (Lucas *et al.* 1998; Jang & Lucas 2005). Under the Habitats and Species Directive the Derwent is an SAC, for which *L. fluviatilis* and *P. marinus* are designated features. The freshwater-resident brook lamprey *L. planeri* is also present (Whitton & Lucas 1997).

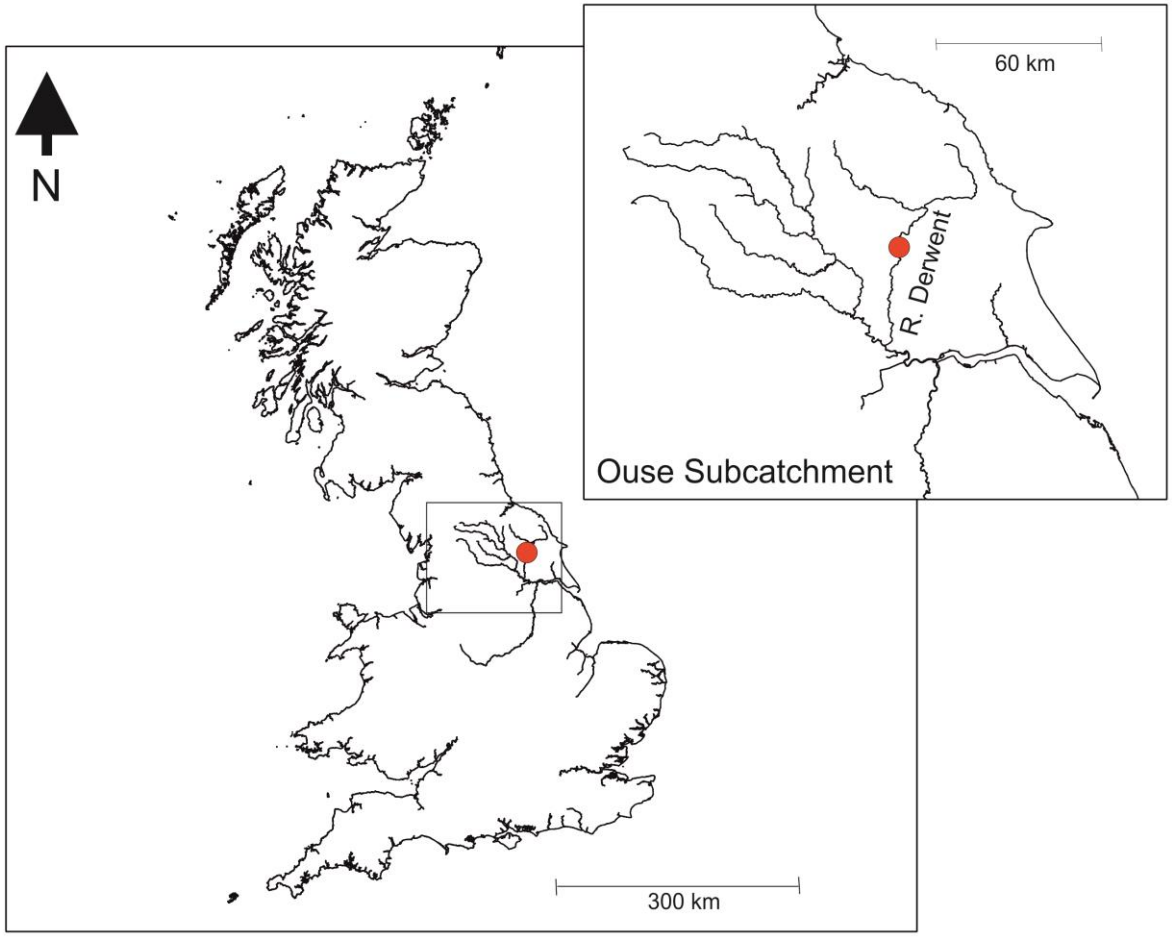



**Figure 2. 1** Map showing the location of Howsham Mill (red circle) on the River Derwent in the Ouse catchment.

This study was carried out at the site of a three-bladed Archimedes screw (Figure 2.2) with a maximum power output 24 kW at Howsham Mill (National Grid Reference SE 496 799, Figure 2.1) which was installed by the Renewable Heritage Trust in 2008. The facility is located at the left bank of an 80 m wide, 1.8 m high, oblique weir with a sloping apron. The turbine has a coarse trash screen with bar spacing of 10 cm, but no fish diversion screen. The turbine's position relative to the weir and river bank topography results in it drawing water from approximately a 4 m wide zone above the turbine and discharging it at the base of the weir on the left bank. A 4 m wide flowing bypass canal exits the river on the left bank, 80 m upstream of the turbine and reconnects with the river approximately 120 m downstream (Figure 2.3).


**Figure 2.2** Hydraulic screw turbine in place at Howsham Mill and schematic of Archimedes screw turbine shown from the side and above (inset; MannPower Consulting).

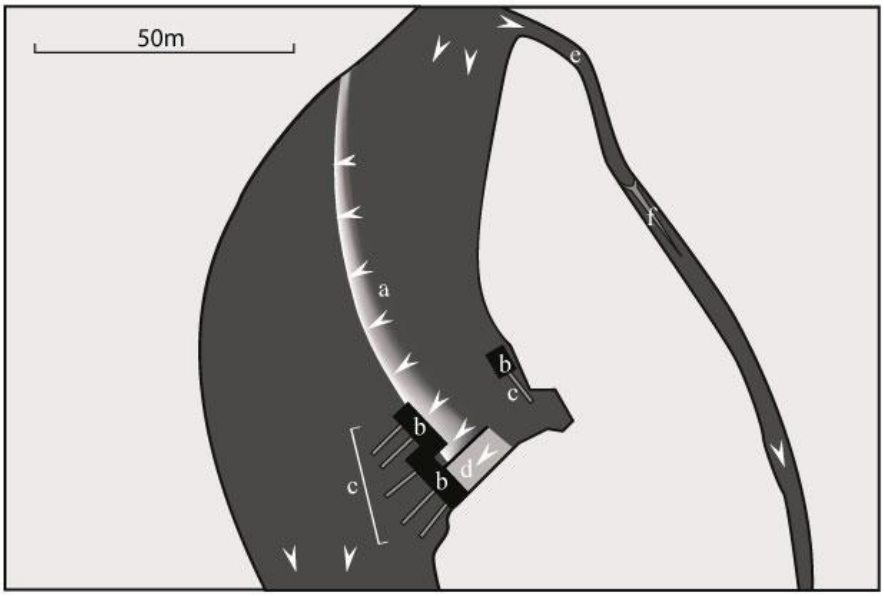



**Figure 2.3** Schematic map of the study site; (a) weir; (b) floating pontoons for drift net deployment; (c) drift nets; (d) hydraulic screw; (e) bypass canal; (f) bypass canal net; arrows, flow direction. The map is drawn approximately to scale, with the exception of some items such as the turbine which have been exaggerated for clarity.

## 2.3 Methods

To assess patterns of abundance of emigrating river lamprey transformers and drifting larvae, drift nets were set in the river channel at Howsham Mill. Since the main emigration period of *L. fluviatilis* transformers is known to be from late winter to early spring (Hardisty *et al.* 1970; Potter & Huggins. 1973), including in the River Ouse catchment that contains the Derwent (Frear & Axford 1991), year-round sampling was not carried out. Sampling was carried out over the periods January to June 2009 and November 2009 to May 2010.

Floating 2 m wide pontoons were placed in the main channel above and below the weir to provide platforms for setting up to six drift nets (Figure 2.4). Flow at the left hand bank margin 10 m upstream of the weir , flow from the turbine, and flow in the main channel, 10-15 m from the left bank and immediately below the weir were sampled (Figure 2.3). Nets were 3 m long, with an opening of 0.50×0.40 m and a mesh size not exceeding 3 mm. The downstream end of the net was weighted, so that it sank towards the bottom. Pilot studies were conducted in January 2009 during which marked transformer (total $n$ =18, 91-118 mm length) and larval (total $n$ =34, 80-122 mm length) lamprey were placed in the sampling nets after dusk, over three trials (2-14 hour duration) to assess the retention capacity of the nets. At net-entrance water velocities exceeding 0.2 m $s^{-1}$ all individuals were retained alive in the drift nets. The precise positioning of the nets varied between sampling dates and was adjusted according to the flow regime on the day, so that each net was typically set in flow exceeding 0.3 m $s^{-1}$. The nets were set with the top edge less than 0.1 m below the water surface and so fished within 0.5 m of the surface in depths of 1-2 m. A larger net with a 4 m long cod end and two heavily weighted lateral wings, each measuring 4 m, and with a mesh size of 3 mm was set across the full width (*c.* 4 m) and depth (*c.* 0.7 m) of the bypass canal to capture downstream-moving fishes at that location.

**Figure 2.4** Floating pontoons located downstream of the weir and the turbine showing some drift nets deployed below the turbine.

Sampling was conducted monthly within the two study periods and nets were fished for day and night periods (checked early in the morning and early in the evening), usually consisting of two nights and the intervening day period. A total of 132 (0.5×0.4 m) net samples were taken by night and 50 by day over the full study period. Eight canal net samples were taken by day and 19 by night. All captured ammocoetes, transformers and adults were identified (Potter & Osborne 1975; Gardiner 2003) and measured under anaesthesia (MS-222, 0.1 g $L^{-1}$) and allowed to recover fully before being returned to the river. As *L. planeri* and *L. fluviatilis* cannot be distinguished externally at the ammocoete stage they were recorded as *Lampetra* spp.

Flow velocity measurements were taken (using a Valeport electromagnetic flow meter, model 801) at the mouth of each net when they were set and again when they were emptied over the period from December 2009 to March 2010. From these data, the

volume sampled by each net and the numbers of lamprey caught per standard volume of water sampled were calculated. River discharge data, at 15-minute intervals, were obtained from a gauging station 5 km downstream. Turbine discharge was also recorded. This proportion of river flow passing through each net was calculated allowing estimation of the numbers of transformers passing through the river over sample periods and the fraction that could pass through the turbine.

To explore the influence of time of year, and river flow, on the catch rate of both ammocoetes and transformers a generalized linear model (GLM) using a Poisson distribution (as the response variable is count data) was implemented in R (R Core Team, 2014). Too few data were available to compare the effect of months, so to increase sample size and to maintain an ecologically meaningful temporal scale, the data were divided into seasons: Winter (December –February); Spring (March – May); Summer (June – August); Autumn (September – November). No data were collected during the summer season. Season was entered as a factor and flow was entered as a continuous variable. The response variable was the number of lamprey caught per evening. Prior to the application of the GLM, a Pearson's product-moment correlation test was also carried out on the data.

### 2.3.1 Experimental passage through the turbine

Some lampreys captured in drift nets exhibited local dermal haematoma and/or fin abrasion or were dead in the nets downstream of both the turbine and weir (control). Therefore, it was not possible to infer impact of passage through the turbine, so direct testing was necessary. Preliminary tests with dead and live lamprey larvae and transformers introduced immediately above the turbine showed that both categories

were recaptured in drift nets (set as described in Section 2.3) placed 4 m from the turbine outfall. Subsequently, a total of 131 lampreys, consisting of 42 river lamprey transformers, 88 *Lampetra* spp. ammocoetes exceeding 80 mm and one adult brook lamprey, were captured by electro-fishing and marked, under light anaesthesia, with an Elastomer Visible Implant under the skin in the caudal third of the body. The lower size limit was chosen to facilitate marking and ensure retention in the net. Lampreys were measured and body condition was assessed for any damage. On recovery from anaesthesia, all individuals were assayed for normal anguilliform swimming behaviour, in a white (to provide high contrast), water-filled tray, while viewed from above. All swam normally and were without damage. Six drift nets were placed, side by side, 4 m below the turbine spanning the main outflow and its periphery. Complete sampling directly at the outflow was not possible due to the intense flow. At dusk lamprey were released immediately above the hydraulic screw. The nets were checked after 30 minutes and each recaptured individual was measured and visually assessed for any discernible changes to body condition and swimming ability. A swimming impairment was defined as any notable deviation from normal sinusoidal undulatory swimming movement.

Data were analysed for deviation from normality and heteroscedeaticity of variance. Appropriate non-parametric tests (Mann-Whitney U and Kruskal-Wallis) were applied using SPSS (ver. 17.0; SPSS 2008) to test the significance of variance due to a non-normal distribution of the data.

## 2.4 Results

### 2.4.1 Diel and seasonal abundance

Totals of 263 river lamprey transformers and 228 *Lampetra* spp. ammocoetes, as well as six adult brook lamprey (*L. planeri*) were caught in the drift nets. In the main channel catch rates (mean and SE) were 1.86 ± 0.53 transformers per net period and 1.08 ± 0.14 ammocoetes per net period by night, and 0.08 ± 0.04 transformers per net period and 0.14 ± 0.04 ammocoetes per net period by day. Night catches in the main channel were significantly higher than daytime catches for transformers (Mann-Whitney test, $U$ = 1959, $P < 0.001$) and ammocoetes (Mann-Whitney test, $U = 1917.5$, $P < 0.001$) with 24-fold and 8-fold greater differences respectively. In the canal, the transformer catch rate by day did not differ significantly from that at night, (Mann-Whitney test, $U = 44$, *ns*) but only eight transformers were caught over 8 day- and 19 night-sampling periods. However, the ammocoete catch rate in the canal by day was significantly lower than that at night (Mann-Whitney test, $U = 25$, $P < 0.01$), with a total of 84 caught by night and four caught by day. Subsequent data presented are night-time catches only.

Ammocoetes were caught in all months, with a peak in mid-winter (Figure 2.5a) while *L. fluviatilis* transformers were caught from November to May, with peak catches from December to April (93% caught over this period) in the main channel (Figure 2.5b). Catch rates in the main channel varied significantly between months for both transformers (Kruskal-Wallis test, $H(7) = 55.5$, $P < 0.001$) and ammocoetes (Kruskal-Wallis test, $H(7) = 43.7$, $P < 0.001$).

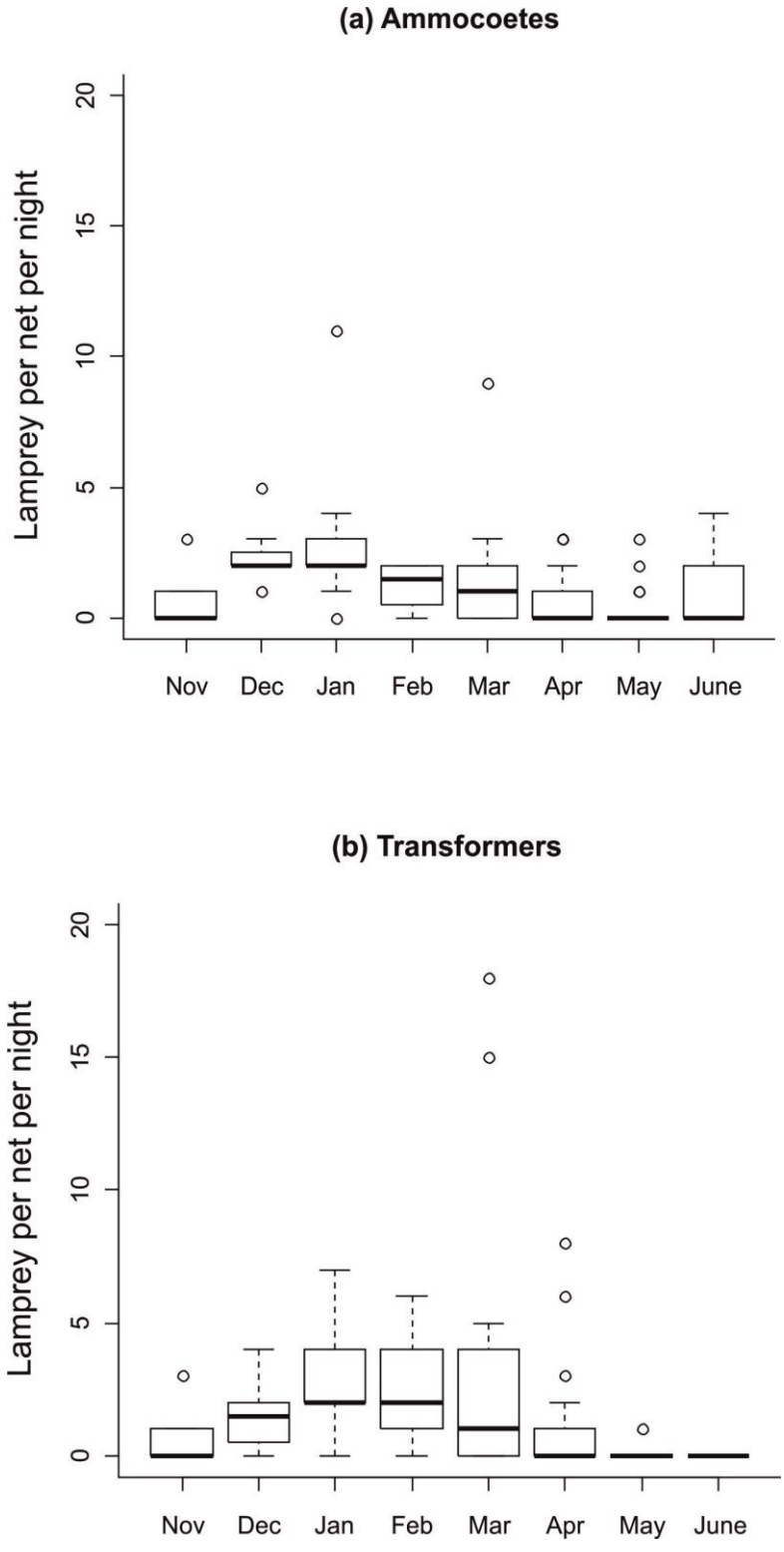



**Figure 2.5** Seasonal distribution of (a) *Lampetra* spp. ammocoete and (b) *Lampetra fluviatilis* transformer catch per net night over the whole sampling period in the main channel. Boxes show median and quartiles, whiskers show the 10th and 90th percentiles, outliers shown as circles.

The Pearson's product-moment correlation test revealed that there was a significant correlation between river flow ($m^3$/sec) and the number of ammocoetes caught per night (t = 6.60, df = 11, $p$ = <0.001, correlation coefficient= 0.89, Figure 2.6a), but no significant correlation was found between river flow and the number of transformers caught per night (t = 2.09, df = 11, $p$ = 0.06, correlation coefficient= 0.53, Figure 2.6b). A significant correlation was also present between season and both ammocoete (t = -2.53, df = 11, $p$ = 0.028, correlation coefficient = -0.61, Figure 2.6c) and transformer (t = -2.26, df = 11, $p$ = 0.045, correlation coefficient= -0.56, Figure 2.6d) catch rates.

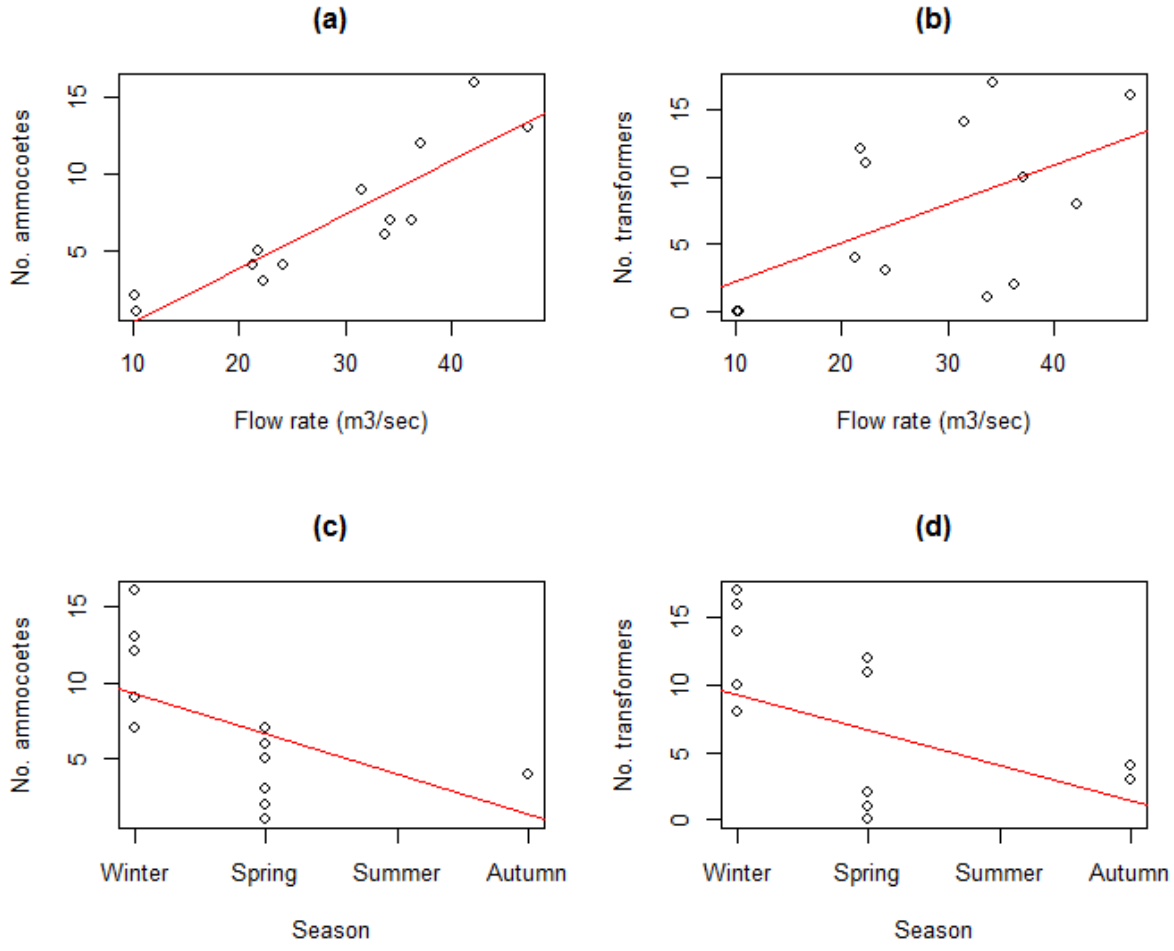



**Figure 2.6** Pearson's product-moment correlation tests showing correlation between a) Flow rate and ammocoete catch b) flow rate and transformer catch, c) season and ammocoete catch and d) season and transformer catch.

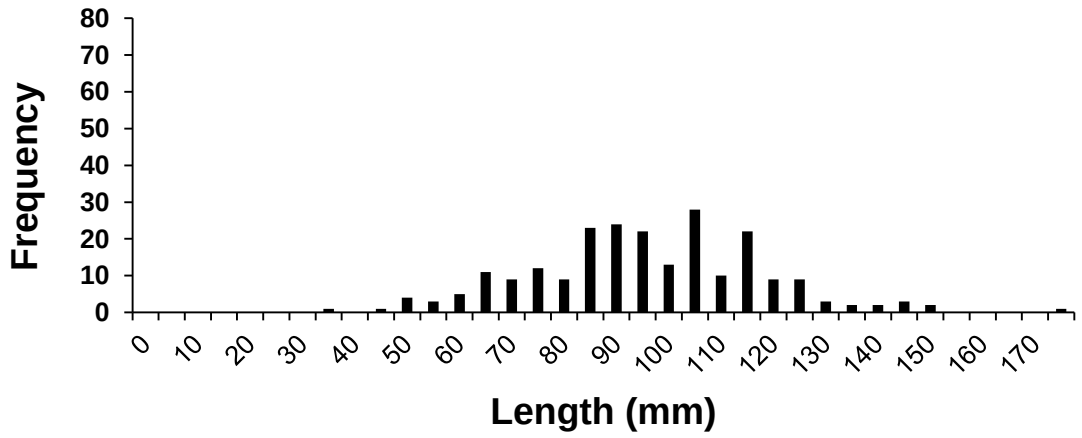
The GLM's for ammocoetes and transformers showed a similar pattern, where ammocoete catch was found to be strongly influenced by flow ($p < 0.01$, Table 2.1); however, there was no longer any evidence to suggest that season was an influential factor. Whereas for transformers, the converse was true; the flow did not influence transformer catch but season significantly affected catch rate with the highest catches occurring in winter (Table 2.1). The models were checked and there were no concerns regarding goodness of fit (Appendix B).

**Table 2.1** GLM Coefficients for the ammocoete and transformer models, both models contained Season and flow. The estimate and the standard error of the estimate are presented, along with the p value.

| | **Estimate** | **Std. Error** | ***P*** |
|---|---|---|---|
| **Ammocoetes** | | | |
| Intercept | 0.68234 | 0.63896 | 0.29 |
| Spring | -0.39915 | 0.3179 | 0.21 |
| Autumn | -0.3114 | 0.46334 | 0.5 |
| Flow | 0.04471 | 0.01566 | 0.004 |
| **Transformers** | | | |
| Intercept | 2.437314 | 0.573847 | <0.001 |
| Spring | -1.04586 | 0.326403 | 0.001 |
| Autumn | -1.2597 | 0.459911 | 0.006 |
| Flow | 0.003315 | 0.014531 | 0.82 |

Ammocoete lengths ranged between 30 mm and 175 mm (Figure 2.7a). Ammocoetes displayed a wide range of sizes but the majority of individuals caught were between 85 mm and 115 mm. Length of transformers varied less than that of ammocoetes and ranged from 75 mm to 124 mm but most individuals ranged between 95 mm and 100 mm (Figure 2.7b). The mean lengths for transformers and ammocoetes were 98.9 mm and 93.7 mm respectively.

(a) Ammocoetes




(b) Transformers

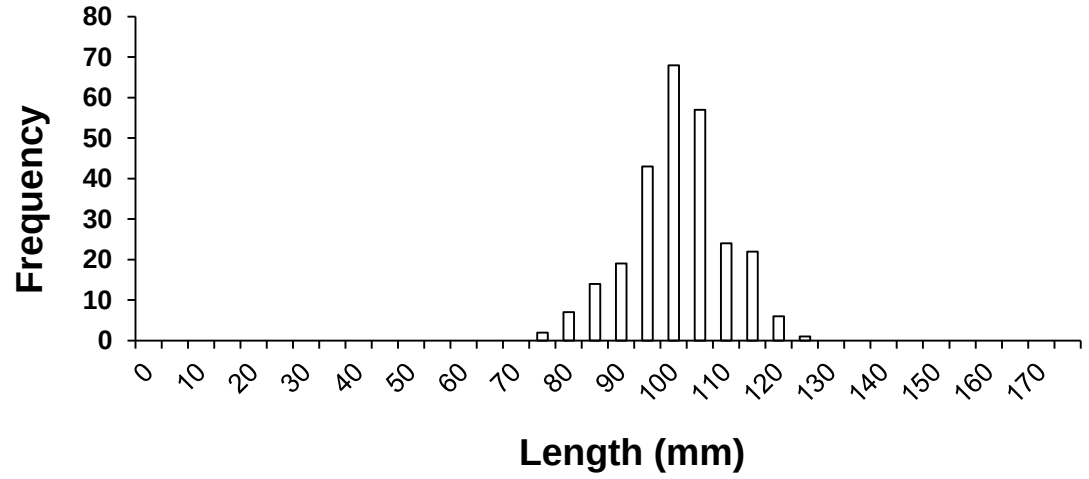



**Figure 2.7** Length-frequency distributions of (a) all *Lampetra* spp. ammocoetes (*n* = 228) and (b) all *Lampetra fluviatilis* transformers (*n* = 263).

### 2.4.2 Risk of turbine entrainment

An estimate of the numbers of migrating transformers that passed through the turbine on several sampling dates was derived from estimates of densities of lamprey per unit volume of water flow and from the fraction of river flow passing through the turbine. All data below are expressed as mean ± SE and are derived from seven separate sampling nights between December and March 2010. Combined, nets sampled 1.96 ± 0.2% of estimated main river flow (36 ± 4.1 $m^3 s^{-1}$) volume at the weir.

By comparison, 0.3 ± .01 $m^3 s^{-1}$ (about 1% of river flow) passed through the canal. Assuming random distribution of lampreys across the river channel in proportion to flow, and that drift behaviour dominates, the estimated number of emigrating transformers passing through the main channel was 677 ± 96 individuals per night and the proportion of water (and hence, entrained transformers) through the turbine was 6.13 ± 0.79%.

### 2.4.3 Experimental passage through the turbine

Out of 131 lampreys that were passed through the turbine, 50.4% were recaptured by drift nets immediately downstream of the turbine within 30 minutes of release (Table 2.2). There were no mortalities but one transformer exhibited swimming impairment (1.5% of all lamprey recaptured).

**Table 2.2** Percentage of marked lamprey introduced to the screw turbine recaptured and effects of the turbine on these. *na* = not applicable.

| Life stage | Number released | % recaptured | % mortality | % swimming impairment |
|---|---|---|---|---|
| Ammocoete | 88 | 46.6 | 0 | 0 |
| Transformer (*Lampetra fluviatilis*) | 42 | 59.5 | 0 | 2.4 |
| Adult (*Lampetra planeri*) | 1 | 0.0 | *na* | *na* |
| Total | 131 | 50.4 | 0 | 1.5 |

### 2.4.4 Distribution within the channel

The abundance of ammocoetes and transformers standardised with respect to volume of flow sampled were compared across four categories of flow; marginal, upstream of the turbine; main flow below the weir; main flow below the turbine; and in the canal (Figure 2.8). There was no significant difference in the number of ammocoetes caught per standard volume sampled in each of the above defined flow categories. However, there was a significant difference in the number of transformers caught in each flow category (Kruskal-Wallis test, $H$ (3) = 23.7, $P < 0.01$). The capture rates of transformers in the canal and in marginal areas were significantly lower than in the main flow downstream of the weir and downstream of the turbine (Mann-Whitney $U$ with Bonferonni-corrected significance at $P = 0.0083$).

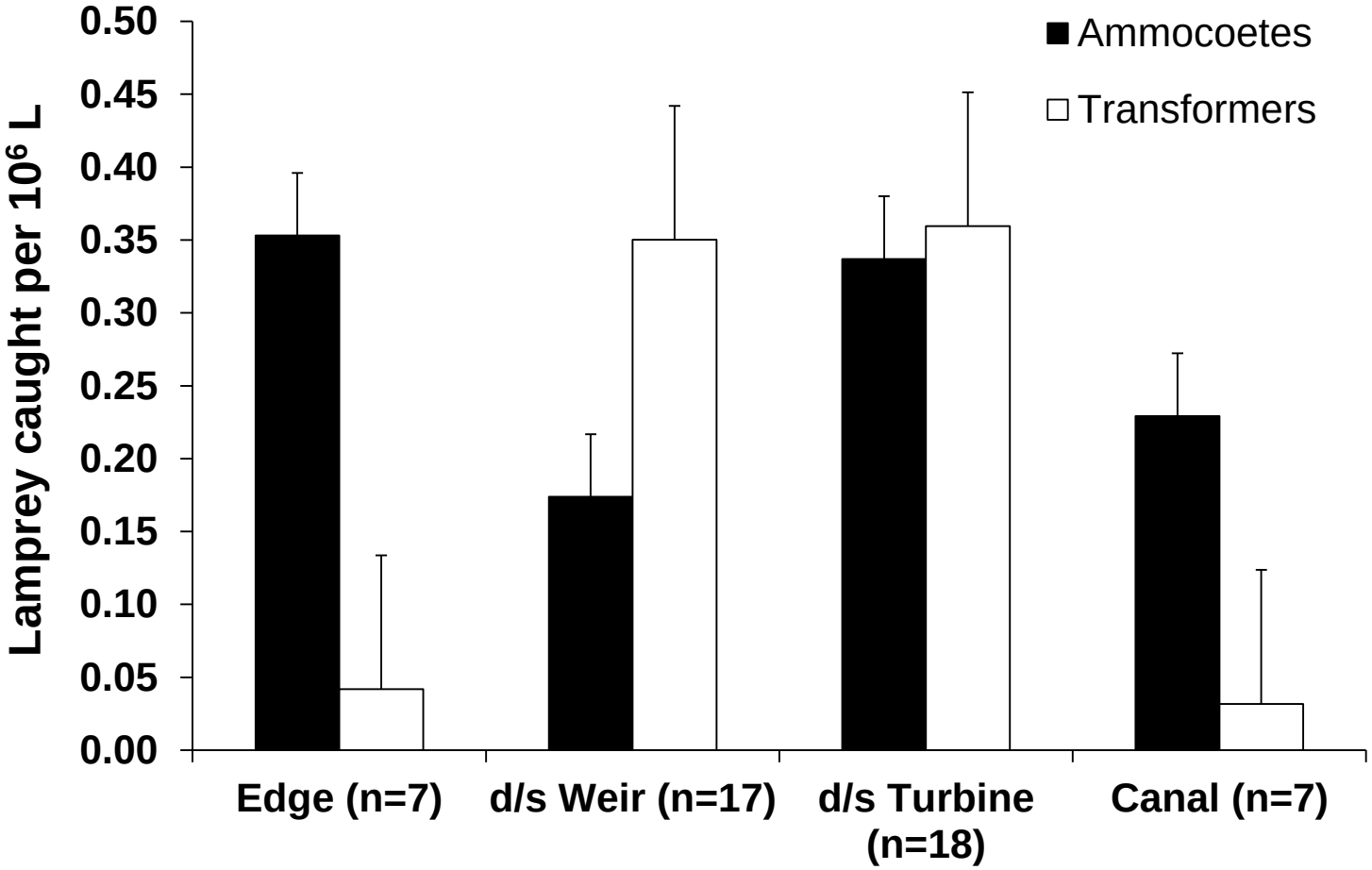



**Figure 2.8** *Lampetra* spp. ammocoete and *Lampetra fluviatilis* transformer catches in differing flow habitat types expressed as mean and standard error of the number caught per $10^6$ L of water sampled. Data are night-time catches for December 2009 to March 2010 combined, the main emigration period for transformers. Abbreviation: d/s = downstream.

## 2.5 Discussion

This study demonstrates that in the River Derwent, *Lampetra* transformers and larvae occur in the water column over extended periods of the year and so are susceptible to entrainment by run-of-river hydropower, but that a single Archimedes screw caused low rates of acute damage to transformers and larvae passed through it unharmed.

### 2.5.1 Diel and seasonal abundance

Night catch rates for both ammocoetes and transformers were significantly greater than day catch rates. Ammocoetes and transformers exhibit strong negative phototaxis and previous studies also suggest that lamprey activity is principally nocturnal (Potter & Huggins. 1973; Potter 1980; Quintella *et al.* 2005; Dauble *et al.* 2006). It is therefore logical that more downstream movement, by either active (Quintella *et al.* 2005; Kirillova *et al.* 2011) or passive means, occurs during low-light conditions. Long (1968) reported 62% of downstream migrating *E. tridentatus* passed the Dalles Dam (Columbia River, N. America) powerhouse at night. During daylight, transformers either burrow (like ammocoetes) or move into protected areas that provide cover (Kelly & King 2001). Strongly nocturnal behaviour in migrating lampreys has been interpreted as an anti-predator tactic (Sjöberg 1989). Similar to lampreys, adult eels (*Anguilla australis* and *Anguilla dieffenbachii*) in New Zealand typically migrate downstream at night (Boubée *et al.* 2001). A similar result was encountered in a study conducted in the Netherlands, which found that 63% of Atlantic eels that passed through a hydropower turbine did so during the first five hours of the night. Interestingly, on free-flowing sections of the river, only 35% of eels passed during the same period at night indicating significant behavioural changes caused by the presence of the turbine (Winter *et al.* 2006).

Analysis of monthly catches between November and June showed significant variations in the catch rates of both transformers and ammocoetes in the main channel. GLM's revealed that ammocoete catch was found to be strongly influenced by flow however; there was no longer any evidence to suggest that season was an influential factor. The GLM revealed that for transformers, flow did not influence transformer catch, however, season did significantly affect catch rate with the highest catches occurring in winter which is concurrent with the peak period for transformer migration described elsewhere (Hardisty *et al.* 1970; Potter & Huggins. 1973), including for the Yorkshire Ouse (Frear & Axford 1991), of which the Derwent is a tributary. However, the overall period of river lamprey emigration in this study was longer than described in those literature sources. In UK rivers where *P. marinus* are abundant, peak emigration timing is in late autumn (Kelly & King 2001), extending the key period of impingement risk for emigrants if both *L. fluviatilis* and *P. marinus* are considered.

Ammocoetes were caught in all months sampled with a peak in mid-winter. Large size classes dominated catches, probably reflecting size selection by the mesh size employed. Ammocoetes longer than 120 mm are more likely to be *L. planeri* than *L. fluviatilis* (Gardiner 2003). The GLM revealed that ammocoete catch was found to be strongly influenced by flow however; there was is no evidence to suggest that season is an influential factor. Downstream drift in ammocoetes takes place most intensively in recently emerged lamprey larvae (Applegate 1950; Hardisty & Potter 1971a; Kelly & King 2001) but ammocoetes of differing age groups are also known to exhibit downstream movement before metamorphosis (Hardisty & Potter 1971a; Potter & Huggins. 1973; Potter 1980; Sjöberg 1980). The significance of these downstream

movements is that it redistributes larvae within a river system and disperses them to the most suitable habitat (Potter 1980). The downstream movement of larvae has previously been found to be season- and temperature-dependent (Kelly & King 2001), which may be coupled with higher winter flows that displace ammocoetes residing in unstable silt beds (Hardisty & Potter 1971a). Migratory behaviour of transformers has, however, in the past, also been shown to be influenced by a marked increase in freshwater discharge (Potter 1980). Pirtle *et al.* (2003) found that a substantial proportion of *E. tridentatus* ammocoete (and transformer) movement occurred during high flows, possibly associated with sediment scour, but movement occurred in other periods also.

The timing of the peak period of emigration and drift should be taken into account when considering how best to reduce the impacts of entrainment and impingement on lamprey. The running of turbines primarily during the day at sensitive sites and seasons could protect emigrating lamprey effectively. Turbines on the Columbia and Snake River systems (USA) are operated within 1% of peak efficiency during the juvenile and adult salmonid migration season to reduce injury and increase fish survival rates (Čada 2001; Ferguson *et al.* 2006). However, this association is controversial as peak efficiency encompasses a wide range of discharge levels, and therefore the zone of operating conditions within 1% of peak efficiency will probably also encompass the maximum turbine passage survival (Mathur *et al.* 2000; Skalski *et al.* 2002). So although this system may be a useful guide for managing turbine operating conditions, there can be an appreciable difference between peak observed survival and the survival at peak turbine operating efficiency (Skalski *et al.* 2002). Where 'fish friendly' turbines can be demonstrated to have very low impacts on fish, shut-down periods may be unnecessary.

### 2.5.2 Turbine entrainment

The proportion of water (and potentially, entrained transformers) passing through the turbine was 6.13 ± 0.79% during the main emigration period, with the highest estimated numbers of transformers passing through the turbine in late January and early February. Throughout the main transformer emigration period from December to March (based on this study, as well as those cited earlier) on a given night the number of migrants passing through the turbine, and potentially at risk, ranged from 21 to 56 individuals and would equate to several thousand over the main emigration period at this site. Losses may be caused by actual damage incurred on passing through the turbine or indirect effects, such as increased predation of disorientated individuals. It is possible that lamprey predators such as grey herons (*Ardea cinerea*) and other birds, otters (*Lutra lurtra*), or predatory fish, would concentrate in turbine outflow areas. Local aggregations of predatory fishes have been identified downstream of turbine outflows in other studies (Lucas & Baras 2001).

Nearly 60% of transformers and 47% of ammocoetes were recaptured within 30 minutes of release, most within 15 minutes. Incomplete recapture was most likely due to incomplete sampling of the turbine flow. There were no mortalities but one transformer (1.5% of all recaptures) exhibited swimming impairment. This impairment was assumed to be due to passage through the turbine as preliminary tests showed that transformers and ammocoetes retained in drift nets for short periods of time (i.e. 2 hours or less) did not exhibit any signs of altered physical appearance or swimming behaviour. For the lampreys passed through the turbine, no external damage or haematoma was observed, but lampreys were not subsequently retained to determine any delayed effects.

Entrainment of *L. fluviatilis* and *L. planeri* can occur at varying stages of their life cycle (Williams & O'Keeffe 2008). However, due to the lack of substantive downstream migration in adults (due to the semelparous nature of their breeding), entrainment is more relevant to downstream moving ammocoetes and transformers (Frear & Axford 1991). Lucas *et al.* (2007) found that only 1.2% of ammocoetes were damaged in a small-scale run-of-river hydroelectric power station with a Kaplan turbine on the River Tay, Scotland. This suggested only a minimal impact to larval lamprey. At a Ritz-Atro hydraulic screw in Germany, 4.4% of teleost fish experimentally passed down the screw were injured during passage. This was most likely caused by contact with the metal edges at the leading edge of the helical blades (Spah 2001). Merkx and Vriese (2007) found no damage to non-lamprey freshwater fish species that passed through an Archimedean screw at Hooidonkse Mill, the Netherlands, and Kibel (2007) found entrained salmonids exhibited only minor (1.4%) scale loss at a screw turbine on the River Dart. Passage through a hydraulic turbine by the European eel (*Anguilla anguilla*), with a similar body shape to lampreys, showed low rates of damage; zero and 0.64% (Spah 2001; Kibel *et al.* 2008).

In contrast to the Archimedes screw turbine, a more typical Kaplan type turbine in the Netherlands was found to cause at least 9% mortality (figure calculated from recaptures so estimation of actually mortality was closer to 16-26%) to silver eels (*Anguilla anguilla*) passing two hydropower turbines on their downstream migration (Winter *et al.* 2006). However, Schmalz (2010) found considerably greater rates of damage to a wide range of fish species that passed down hydraulic screws and demonstrated damage to the blades, possibly caused by gravel. Such damage could increase the severity of strike impacts to fish over extended operational periods, although rubber covers to blades (Kibel 2007) may be effective in reducing such effects.

These findings, including the current study, support the suggestion of O'Keefe and Turnpenny (2005) that very small fish, including larval and juvenile lamprey, are likely to pass through low-head turbines, especially hydraulic screw designs, without substantial damage. They also support the view of Moursund *et al.* (2003) that juvenile lampreys are relatively robust in anatomy and physiology to turbine passage. It seems that juvenile lampreys can tolerate turbine passage but may, however, be more susceptible to screen impingement (Moser *et al.* 2013). For larval and juvenile lampreys the impact of fine screens are likely to be greater than passage through the turbine itself, which has resulted in the recent development of specific bypass screens at dams that allow safe lamprey passage (Moursund *et al.* 2003). Traditional woven wire mesh screens at hydropower dams in the Columbia River basin (USA) have 7 mm diagonal openings that can entrap juvenile lamprey, laboratory tests have indicated that this must be increased to 11 mm to allow safe passage for Pacific lamprey (*E. tridentatus*) ammocoetes and transformers (Moser & Vowles 2010). Gap size within screens to divert migrating adult Pacific lampreys at these hydropower facilities has also been considered (Moser *et al.* 2008). Alternative methods of diverting downstream moving juvenile lampreys away from turbines, such as pulsed direct current, are also currently being explored (Johnson & Miehls 2013). Nevertheless a wider range of studies of low-head turbine impacts on fishes, including those examining chronic, sub-lethal effects are needed (Cooke *et al.* 2011).

There was no significant difference in ammocoete catches, standardised to volume sampled, in differing parts of the channel. This suggests that ammocoetes captured were drifting downstream and behaving essentially as passive particles. Thus numbers of ammocoetes entrained into turbine flow are likely to be directly related to the proportion of flow. Little behavioural avoidance is likely to be achieved by any inflow

modification in the vicinity of the turbine entrance. In contrast, the data provide some evidence to suggest that transformers avoid edge and lateral water off-take. Higher catch rates of transformers per unit volume sampled occurred in the areas of greater flow which passed through the turbine and over the weir. This may be due to differences in our catch efficiency, or by a non-random distribution of transformers, mediated by behaviour. Transformers have well developed sensory systems and their downstream migration has been linked to high water flows (Potter 1980). It is therefore likely that river lamprey transformers preferentially move along main flow routes and orientate away from the areas near the river's edge. Lateral or slack-water off-takes may represent less of an entrainment risk to river lamprey transformers than water off-takes from the main current.

Other behaviours exhibited by downstream moving lampreys can also affect the success of their downstream migration at hydropower facilities. Factors such as the mere presence of a structure within a river (such as a turbine or a weir) can reduce the likelihood of lampreys moving past these structures due to altered behaviours at these sites. For example, radio-tagging studies have shown that fish can be reluctant to move downstream over, or through, regulating structures, and can often return upstream when confronted with a weir (Haro *et al.* 2000; Behrmann-Godel & Eckmann 2003). When confronted with a structure such as a hydroelectric dam, downstream migrant American eels (*Anguilla rostrata*) have been shown to spend time searching for an unobstructed pathway downstream. If they are unable to find a suitable pathway, a tendency to return upstream is exhibited, often to a location where they were residing previously (Haro *et al.* 2000). Similarly, European eels were found to be distributed in proportion to the river discharge until they approached the entrance to a hydropower

turbine, where they altered their behaviour and showed stationary and recurrent behaviour (Jansen *et al.* 2007).

Any abstraction or diversion of water from rivers, lakes estuaries or the sea carries a risk of harm to fish that are present (Turnpenny *et al.* 1998). Archimedes screw turbines appear to have little effect on lamprey transformer and ammocoete passage. The cumulative impacts of turbines, even 'fish-friendly' ones such as Archimedes screws must, however, be considered. Cumulative impacts of multiple hydropower stations, dams or small weirs are evident across a wide range of fish taxa, including lampreys (Williams *et al.* 2001; Moser *et al.* 2002; Gowans *et al.* 2003; Lucas *et al.* 2009). Whilst single hydropower schemes may have relatively minimal effects on fish communities, the cumulative effects of more than one scheme in a river, or catchment, could potentially be more ecologically damaging. Cumulative impacts could include, delays in fish migration, mortality at impoundments, losses of fish spawning and larval habitat, blockage of migration routes, effects on invertebrate and plant communities, changes in overall river hydrology, and increased predation due to higher concentration of individuals gathering above and below hydropower schemes.

Even where the effects at one site or design are minor, future developments need to take into account cumulative within-catchment impacts as well as site-specific impacts. For example, even if an individual hydropower site causes just a 2% mortality rate, the cumulative impact to a cohort passing six successive sites is a reduction in escapement to a maximum of 88.6%. Yet there are few examples of catchment-wide planning for cumulative impacts of small-scale hydropower (e.g. Environment Agency 2010). Small-scale hydropower in higher order river channels generally has greater potential to affect

diadromous fishes, including lamprey. It is therefore advisable to carefully limit the number, types and locations of small-scale hydropower facilities. In this respect, placing hydropower facilities within identified SAC's for lampreys, can only increase the potential impact on downstream moving lampreys due to their presence there. The development of 'fish friendly' turbines could lead to the rapid multiplication of low-head power generation sites within river systems, enhancing renewable power contributions. However, further research is needed to assess wider and longer-term impacts; for example, indirect effects of increased predation risk. The Environment Agency (2010) advises that hydropower development in England and Wales should be concentrated in severely degraded areas, in the context of the European Water Framework Directive.

I might here speak of many other fish whose shape and nature are much like the Eel and frequent both the Sea and fresh River; as name the Lamprey, the Lampern and the Lamprel..... and might also tell in which esteem many of them are for the curiosity of their taste. But these are not proper to be talked of by me, because they make us Anglers no sport, therefore I will let them alone as the Jews do to whom they are forbidden by their Law.

— *Izaak WaIton*, The Compleat Angler I653.

The river has taught me to listen; you will learn from it, too. The river knows everything; one can learn everything from it.

— *Herman Hesse,* Siddhartha.

# Chapter 3: Phylogeography and demography of the European lamprey species pair *Lampetra fluviatilis* and *Lampetra planeri* as inferred from mitochondrial DNA

## 3.1 Introduction

De Candolle (1820) was the first to propose that the current geographical distribution of living organisms is dependent upon both ecological and historical parameters. In this context, historical parameters, such as the effects of glaciation on the distribution of European species, have been explored in detail (Hewitt 1996). It is known that during both the Pleistocene (*c.* 2,588,000 to 11,700 years ago) and Holocene epochs (11,700 years ago to present), extant species went through many range contractions and expansions. This was characterised by extinctions, or displacement, of northern populations when the temperature decreased, followed by a northward expansion from refugia in the south once temperatures began to rise. It is possible that many populations within northern latitudes went extinct, or may have suffered successive bottlenecks that could have subsequently led to loss of genetic diversity. Many scenarios could have occurred throughout this tumultuous period that would have had profound effects on the biodiversity and distribution of populations today. With the development of modern molecular methods, it is now possible to explore this further, by examining the genetic diversity of extant populations, and making inferences on past movements, expansions and contractions of populations.

The European river lamprey (*Lampetra fluviatilis*) and European brook lamprey (*Lampetra planeri*) are considered to be 'paired' or 'satellite' species whose larvae cannot usually be differentiated morphologically (Zanandrea 1959; Potter & Osborne 1975; Vladykov &

Kott 1979). The adults, however, display distinct life-histories and become either freshwater-resident (i.e. life-cycle takes place wholly in freshwater) and non-parasitic (*L. planeri*), or anadromous and parasitic (*L. fluviatilis*) (Potter 1980; Schreiber & Engelhorn 1998; Gill *et al.* 2003). This trend is common among the order Petromyzontiformes, and paired species exist in seven out of the ten lamprey genera (Renaud 2011). *Lampetra fluviatilis* is mostly distributed in northwest Europe, from western France and the British Isles to Scandinavia but also exists in parts of Portugal (see Figure 1.2 in Chapter 1, Freyhof & Kottelat 2008a). *Lampetra planeri* occupies a similar range in the freshwaters of northwest Europe with a number of populations also existing in Portugal and Italy (Freyhof & Kottelat 2008b). It is generally accepted that lamprey paired species are closely related, the non-parasitic freshwater species having evolved from a similar form to that of the extant parasitic anadromous lamprey (Zanandrea 1959; Hardisty 1986a; Schreiber & Engelhorn 1998; Youson & Sower 2001; Gill *et al.* 2003; Renaud *et al.* 2009; Docker *et al.* 2012). There has, however, been much controversy about the taxonomic status of paired lamprey species (Docker 2009).

Anadromous lampreys require a freshwater environment to spawn. Therefore, they have the opportunity to disperse and colonise previously unexploited freshwater environments. A shift from an anadromous to a wholly-freshwater life history has occurred repeatedly in Petromyzontiformes (Zanandrea 1959; Vladykov & Kott 1979; Potter 1980). The climatic oscillations of the Quaternary (i.e. Pleistocene and Holocene epochs) cold periods have had a dramatic effect on most organisms in temperate regions (Hewitt 1996), and may have supported the divergence of wholly-freshwater forms by cutting off lamprey populations from the sea or estuaries during periods of glaciation. Similarly, glacial retreat could have opened up new freshwater habitats for

anadromous lampreys (Bell & Andrews 1997; Taberlet *et al.* 1998; Hewitt 1999; Lee & Bell 1999).

*Lampetra fluviatilis* and *L. planeri* are currently classified as separate species based on their distinct life histories and morphological differences (Hardisty & Potter 1971c). Recent studies, however, have contested whether these morphological differences are in fact a reliable way to separate the species pairs. Hume (2013) morphologically assessed adult lampreys from seven populations across Scotland and Ireland; five of which were classified as *L. fluviatilis* and two as *L. planeri* according to current taxonomic keys (Renaud 2011). This revealed no consistent morphometric differences between the two forms, indicating traditional taxonomic techniques do not have the power to separate *L. planeri* from *L. fluviatilis*.

The same study (Hume 2013) used mtDNA sequences to also examine this relationship, and found that independently derived non-parasitic haplotypes differed by very few mutational steps from haplotypes found in parasitic specimens in different geographic regions. Several haplotypes were also found to be shared between non-parasitic and parasitic individuals. These results, therefore, support the idea that *L. fluviatilis* and *L. planeri* are more likely to represent ecotypes of a single species than *L. planeri* is to represent a discrete species, and suggests *L. planeri* be synonymised with *L. fluviatilis* (Hume 2013). Therefore, the debate is still ongoing as to whether *L. planeri* are actually just life-history variants (i.e. ecotypes) of a single polymorphic species, *L. fluviatilis*.

Espanhol *et al.* (2007) offer three main scenarios for the origin of non-parasitic lampreys (NP) such as *L. planeri*. The first is that different NP populations are derived from a single event where anadromous ability was lost i.e. NP populations from differing

locations would form a monophyletic cluster distinct from anadromous parasitic lampreys (P), such as *L. fluviatilis*, that would be contained in a second monophyletic cluster (Figure 3.1a). Secondly, that multiple occurrences of NP may result from independent divergences from P, with the repeated loss of anadromy (Figure 3.1b). NP would thus be polyphyletic, and P would be paraphyletic, with geographically proximal NP and P populations being genetically closer to each other. This may be difficult to separate from the third scenario in which NP and P are different ecotypes of a single polymorphic species (Figure 3.1c).

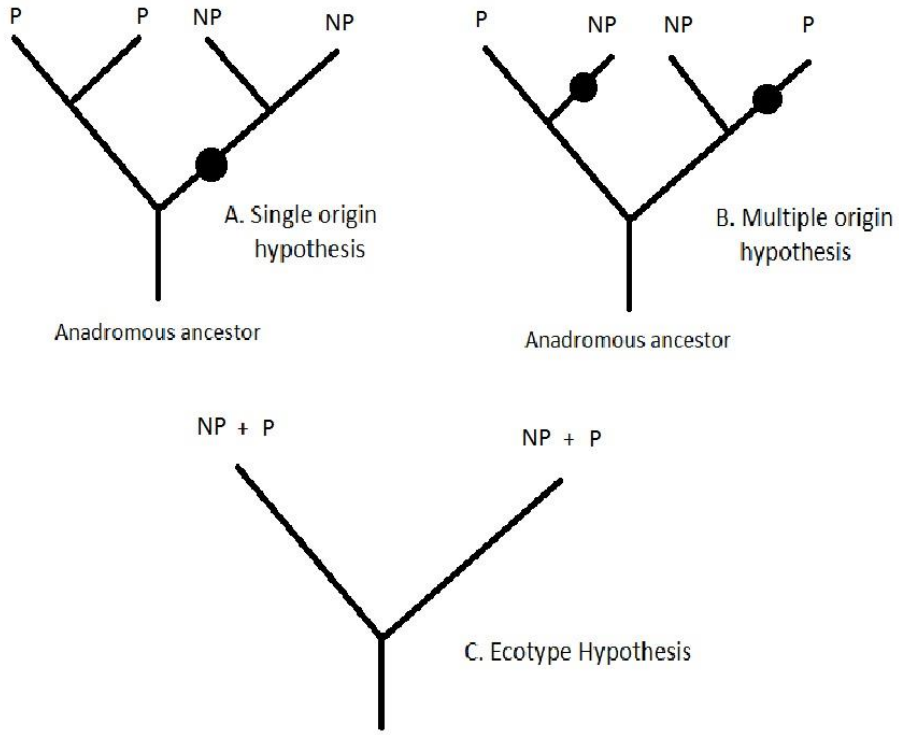



**Figure 3.1.** Alternative hypotheses (a, b, c) of relationships among populations of non-parasitic lampreys (NP) and anadromous parasitic lampreys (P), adapted from Espanhol *et al.* (2007). Filled circles represent loss of anadromy and evolutionary divergence.

Previously, there have been many attempts at using molecular methods to solve the lamprey paired species puzzle. Genetic analysis based on allozymes (Beamish 1985, 1987; Engelhorn & Schreiber 1997; Schreiber & Engelhorn 1998; Yamazaki & Goto 1998) and mitochondrial DNA (mtDNA) markers (Tagliavini *et al.* 1994; Docker *et al.* 1999; Yamazaki *et al.* 2006; Espanhol *et al.* 2007; Blank *et al.* 2008; Lang *et al.* 2009; Pereira *et al.* 2010; Hume 2013) have revealed low, or a complete lack of, genetic divergence between members of paired lamprey species. Espanhol *et al.* (2007) previously used mtDNA to examine the pattern of geographical variation across *L. fluviatilis* and *L. planeri* populations, and concluded this to be consistent with either the hypothesis of parallel speciation (Figure 3.1b) or that *L. planeri* and *L. fluviatilis* are alternate life-history forms of one species (Figure 3.1c). Blank *et al.* (2008) also used mtDNA to explore this relationship, and found *L. fluviatilis* and *L. planeri* to be genetically indistinguishable, indicating either a very recent divergence or ecotypes of the same species. Divergent ecotypes exist in many anadromous fish species, in which a small proportion of individuals are non-anadromous (Gross 1987; Jonsson & Jonsson 1993). Body size at maturation could be the main determinant for lamprey larvae to choose which alternative life-history tactic maximises fitness (Espanhol *et al.* 2007). It is possible, but not certain, that the existence of ecotypes of a single species can lead to speciation due to assortative mate choice between the forms (Salewski 2003).

At one end of the spectrum, geographically isolated populations (i.e. allopatric) can diverge freely, and if population size is small they will be subject to strong genetic drift, which can lead to reproductive isolation and divergence. At the other extreme, however, where populations are sympatric, there are no physical barriers and gene flow may still exist. Nonetheless, there is a body of research to suggest that sympatric speciation can occur under certain circumstances (Via 2001). Speciation, in the presence of gene flow,

is a stepwise process, where processes such as disruptive selection (i.e. selection against the average phenotype in the population), in conjunction with other processes such as assortative mating, can lead to differentiation which progresses as gene flow reduces (Rueffler *et al.* 2006). This type of speciation is generally thought to be difficult, because gene flow will limit population differentiation, consequently preventing the evolution of strong reproductive isolation (Coyne 2007; Nosil 2008). However, due to methodological advances there is increasing empirical evidence for speciation with gene flow (Hey 2006; Niemiller *et al.* 2008). The temporal decrease of gene flow between speciating populations, the factors which constrain gene flow to facilitate divergence, and the generality of the process, are all questions which need to be further investigated (Nosil 2008). The ecological divergence between populations, however, is likely to be of key importance in facilitating speciation in the presence of gene flow (Nosil 2008).

In ecological speciation, the first stage is typically the development of distinct phenotypes within a population (Schluter *et al.* 2001) as a result of adaptation to differing environments which may have become recently available, or were previously inaccessible. If different habitats, or ecological niches, have sufficiently different selection regimes, local adaptation can develop despite substantial gene flow, and reproductive isolation may then develop gradually as a by-product of habitat use and assortative mating (Rice & Hostert 1993). The absence of an intermediate environment (i.e. ecological discontinuity) can also limit gene flow between divergent taxa by causing ecological selection against hybrids (Bush 1994; Hatfield & Schluter 1999; Via *et al.* 2000). Reproductive isolation resulting from ecological divergence between ecotypes in this way has been suggested to be a key driver in the process of speciation in sympatry (Bolnick & Kirkpatrick 2012). Rates of gene flow as low as one migrant per generation are thought to be enough to prevent a loss of genetic diversity through genetic drift

whilst allowing the population to respond to local selection pressures (Mills & Allendorf 1996). Philopatry, or habitat fidelity, further facilitates this type of speciation (Via *et al.* 2000) as this could reduce gene flow enough to allow populations to diverge through local adaptation or by genetic drift.

Glaciation, therefore, may have promoted the divergence of non-parasitic lamprey species by either blocking migration routes and preventing anadromy (and consequently imposing habitat fidelity), or upon de-glaciation making new freshwater habitat and food resources available (Bell & Andrews 1997). It has also been suggested that changes in the environment, in particular the formation of new barriers to migration, or the reduced availability of host fishes (e.g. through over-exploitation), might promote a complete abandonment of adult feeding (Hardisty 1986a). In addition, habitat fragmentation can reduce the size of a population, and consequently the genetic diversity, owing to the inverse relationship between heterozygosity and population size, genetic diversity within smaller populations is lost at a greater rate than it would be in a larger population.

Where few distinguishable morphological features are present to separate species, the molecular approach becomes extremely valuable (Lang *et al.* 2009). Through the analysis of molecular markers, insights are provided into population divergence and dispersal from local to catchment scales. Despite the fact that *Lampetra* spp. larvae are indistinguishable, the associated differences in the life history of *L. fluviatilis* and *L. planeri* should lead to differences in dispersal behaviour, and consequently increases the potential for population structuring and incipient speciation. An approach previously used to evaluate whether genetic population structuring matches a species designation, is

to assess whether divergent populations found sympatrically are more closely related to one another than they are to phenotypically similar allopatric populations (Taylor & McPhail 1999; Wilson *et al.* 2000). This approach was also used within this study and consequently allowed inference to be drawn about population connectivity and evolutionary viability, and has important applications in conservation management (Latta 2008). Genetic analysis of other fish species has been successful in determining recent population structuring, due to postglacial colonisation (Hansen *et al.* 1999) and has also been used to assign fish to their population of origin (Nielsen *et al.* 2001). In this way, molecular techniques can also be used to help extrapolate the evolutionary history of the European lamprey paired species *Lampetra fluviatilis* and *L. planeri*.

## 3.2 Aims

The aim of this study was to examine the genetic diversity and levels of differentiation between several *L. planeri* and *L. fluviatilis* populations from the British Isles and northern Europe using the ATPase 6/8 gene of the mitochondrial genome. The genealogical relationships and geographical distribution of mtDNA haplotypes for *L. fluviatilis* and *L. planeri* from sampling localities at varying latitudes within Europe will then be compared and examined in the context of population expansion into northern Europe from southern refugia. This study also provides information on the mode and timing of divergence of the European lamprey species-pair in northern Europe and attempts to elucidate the demographic history of the species-pair by comparing population genetic relationships.

## 3.3 Methods

### 3.3.1 Sampling

Tissue samples were collected from a total of 108 lampreys across six sites in the British Isles and Europe (Figure 3.2, Table 3.1). This consists of two paired sites (i.e. where *L. planeri* and anadromous *L. fluviatilis* samples were obtained from the same river), the River Dee in Wales and the River Nidd in North-East England, a freshwater-resident population of *L. fluviatilis* from Northern Ireland (Goodwin *et al.* 2006), and an anadromous *L fluviatilis* populations from Belgium. Under the Habitats directive, the River Dee in Wales is a designated Special Area of Conservation (SAC) for which *L. fluviatilis* and *L. planeri* are a qualifying feature, but not the primary reason for the designation of the SAC (JNCC 2007). *Lampetra fluviatilis* and *L. planeri* samples were obtained by either hand-netting, electric-fishing, or trapping migrating or spawning adults as outlined below. Both species were sampled where they were found to be locally abundant during their seasonal spawning and so were, in most cases, captured in the vicinity of their spawning grounds. Only adult lampreys and juveniles identifiable to species were utilised in this study. Adult anadromous, and freshwater-resident *L. fluviatilis*, as well as non-parasitic *L. planeri* can be separated using standard lamprey taxonomic characteristics (Renaud 2011).

*Hand netting*

Both *L. fluviatilis* and *L. planeri* adults from the River Nidd (Table 3.1, Figure 3.2) were caught by hand netting. Probable spawning sites were identified by initial walk-over surveys and consultation with local anglers. Subsequent to identification of active spawning sites, daily checks were made for spawning adults. Spawning lamprey were

then caught by hand-net while they were either nest building or attached to rocks in their nest vicinity. Once caught, individuals were placed in large buckets where they were then measured, and identified under anaesthesia (MS-222, 0.1 g L-1) using a field key (Potter & Osborne 1975; Gardiner 2003) during which time fin clips, taken from the second dorsal fin, were stored in 20 % DMSO saturated NaCl solution (Amos & Hoelzel 1991).

*Trapping*

Traps were set to capture *L. fluviatilis* during their migratory phase in the River Dee (Wales), River Bann (N. Ireland) and the River Scheldt (Belgium) (Figure 3.2, Table 3.1). Trapping, identification, and the taking of fin clips on the River Dee was carried out by I. Davidson and R. Cove who used salmon traps (2 cm bar spaces) which were set below a building at Chester weir creating a dark refuge area which encouraged lamprey to enter. Additional samples were collected by the author by electric-fishing as outlined below. In the River Scheldt, traps (double fyke nets) were set in the main channel (February 2002) by D. Buysse and J. Coeck under the lock-weir complex at Ghent (160 km from river mouth) due to the tendency for *L. fluviatilis* to accumulate in front of the weirs on their upstream migration, traps were set directly beneath the weirs (Buysse *et al.* 2008). D. Buysse and J. Coeck also carried out the identification and collection of genetic samples. The lock-weir complex at Ghent is the first obstruction on the river. Fyke nets were 5 m in length with a mesh size of 8 mm.

Potamodromous (i.e. freshwater-resident in that they migrate within freshwater only) *Lampetra fluviatilis* from the River Bann, which are were collected from Toombridge eel

fishery at Toome, County Antrim, Northern Ireland on their downstream (from Lough Neagh to spawning sites on the River Bann) spawning migration by Claire Goodwin (Goodwin *et al.* 2006). They were trapped from October to November 2002 as by-catch in eel weirs near to outflow of Lough Neagh (Kennedy & Vickers 1993). Eel weirs in the past traditionally consisted of woven sections of willow branches in the shape of a 'V' that are fixed to stakes driven into the bed of a river, the more modern version is a metal frame with four double hooped nets to fish for downstream migrating adult eels (Frost 1950). It is possible that anadromous *L .fluviatilis* do exist in the River Bann, however, the lower River Bann leads from Lough Neagh to the sea (Figure 3.2) and the flow of water out of the Lough into the lower Bann is controlled by flood gates. The eel fishery operates at Toomebridge on the lower Bann just below the Lough entrance. These nets are set to trap downstream migrants and therefore unlikely to trap upstream migrating fish (i.e. upstream migrating anadromous *L. fluviatilis*). Also if there were upstream migrating anadromous lampreys, it would be expected that an eel fishery located further downstream (at Kilrea) would also have a similar number of lamprey catches, however very few (<10 per season) were caught at this location. The reasons for the apparent absence of migratory lamprey in the system are not clear. It is possible that despite its fish pass, a large weir half way down the Lower Bann is impassable to lamprey as has been suggested by Kennedy & Vickers (1993).

*Electric-fishing*

*Lampetra planeri* samples obtained from the River Dee in Wales (Figure 3.2, Table 3.1) were collected by electro-fishing. The following method is generally used for sampling ammocoetes (Harvey & Cowx 2003) but was utilised in this study to capture transformers and adult *L. planeri*. Habitat, which consisted of slow moving water with a

silt/sand substrate and supply of organic detritus, was located and electric fishing was carried out using an Electracatch pulsed direct current (DC) control box powered by a bankside generator (1 KVA) according to the protocol outlined by Harvey & Cowx (2003). The anode was placed under the water surface but not directly on the substrate (~10-15 cm above) and the applied current alternated (on for 20 seconds, off for 5 seconds) for 2 minutes. This on-off cycle draws the buried transformers and adults out of their burrows and into the water column as it does for ammocoetes (Harvey & Cowx 2003). The immobilised lampreys which emerge into the water column were then removed with a fine mesh hand-net and placed into a large bucket. The area was then left to settle for a further 5 minutes before the next fishing commenced. Lampreys were identified using a field key (Gardiner 2003) and fin clips, taken from the second dorsal fin, were stored in 20 % DMSO saturated NaCl solution (Amos & Hoelzel 1991).

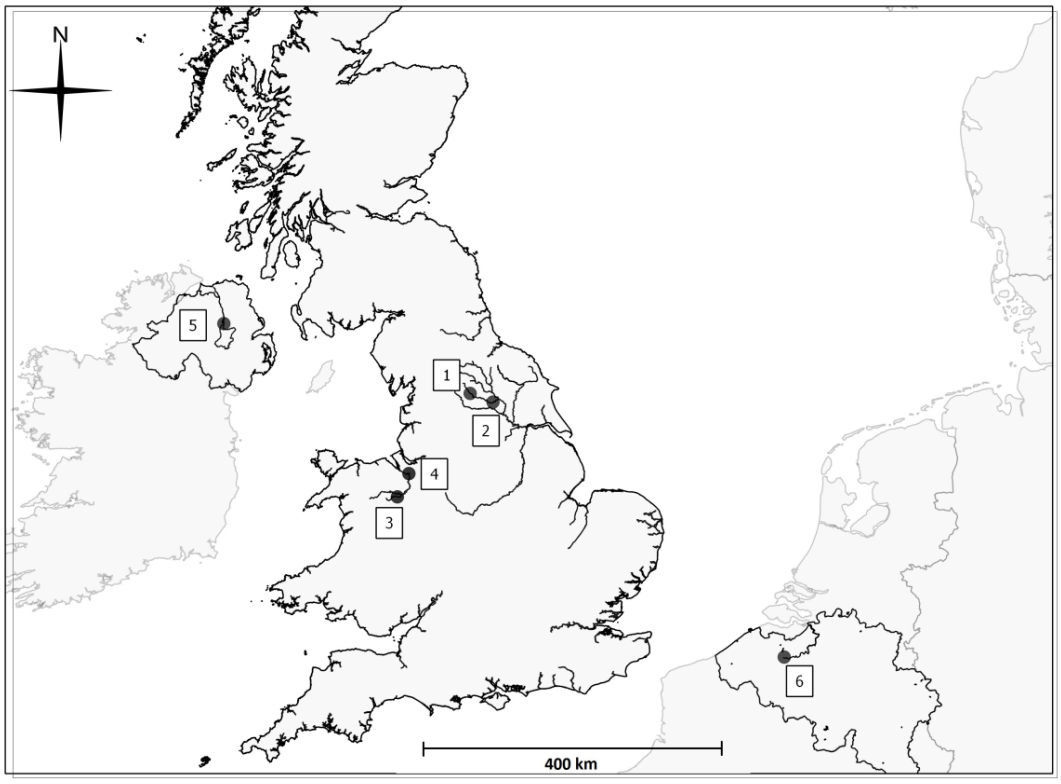



**Figure 3.2** Map showing location of sampling sites 1-6 (see Table 3.1 for details).

**Table 3.1**. Numbers collected and location of origin for all genetic samples of *Lampetra planeri* and *Lampetra* fluviatilis, and by whom they were collected. Where '*L. fluviatilis* (Res)' represents a freshwater-resident population of *L. fluviatilis*.

| Site no. on map | Country | Catchment | River | Latitude | Longitude | *Species* | N | Method of Collection | Collected by: |
|---|---|---|---|---|---|---|---|---|---|
| **1** | England | Humber | Nidd | 54° 4'38.33" N | 1°44'48.81" W | *L. planeri* | 18 | Hand net | F. Bracken |
| **2** | England | Humber | Nidd | 53°58'49.00"N | 1°19'5.99" W | *L fluviatilis* | 17 | Hand net | F. Bracken |
| **3** | Wales | Dee | Ceiriog | 52°55'35.29" N | 3° 4'58.55" W | *L. planeri* | 17 | Electric-fishing | F. Bracken |
| **4** | Wales | Dee | Dee | 53°11'11.37"N | 2°53'14.43"W | *L. fluviatilis* | 16 | Trapping | I. Davidson & R. Cove |
| **5** | N. Ireland | Bann | Bann | 54°45'18.69" N | 6°27'51.06" W | *L. fluviatilis* (Res) | 20 | Trapping | Clare Goodwin |
| **6** | Belgium | Scheldt | Scheldt | 51° 0'25.89" N | 3°45'7.89" E | *L. fluviatilis* | 20 | Trapping | David Buysse & Johan Coeck |
| | | | | | | Total | 108 | | |

### 3.3.2 DNA extraction

DNA was extracted from 108 specimens collected from 6 localities during sampling (Table 3.1). A small part of the tissue sample, approximately (5 $mm^3$) was finely cut using a scalpel. Samples were then incubated overnight at 37 °C in digestion buffer (50 Mm Tris pH 7.5, 1Mm EDTA, 100 Mm NaCL, 1 % w/v SDS) with 30 µl proteinase K (10 mg µl-1). The DNA was then extracted using a standard phenol: chloroform extraction (Sambrook *et al.* 1989). The presence of whole genomic DNA was then confirmed by viewing results on 1.2% agarose gels that were run for 20 minutes, at 120 V and 400 mA, alongside a 1 Kb DNA ladder.

### 3.3.3 Amplification and sequencing of mitochondrial DNA

The primers ATPfor (5'-CCTTTTAAGCTGAAGAAGATGGGTG-3') and ATPrev (5'-TGGTATGCGTGAGCTTGGTGGG-3') (Espanhol *et al.* 2007) were used to amplify 829bp of the mitochondrial gene ATPase subunits 6 and 8. ATPase 6/8 genes were chosen for sequencing so that the dataset could be combined with previous data on European lampreys from Espanhol *et al.* (2007) and Mateus *et al.* (2011). Each 20 µl reaction contained 1.2 µl $MgCl_2$, 2 µl dNTPs (2.0 mM), 0.2 µl of each primer (10mM), 4 µl of GoTaq® Reaction Buffer (Promega), 0.1 µl GoTaq DNA polymerase (Promega), and 1µl of template DNA. Cycle conditions were: initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of; denaturation at 94 °C for one minute, annealing temperature 57.1 °C for one minute and extension at 72 °C for two minutes; followed by a final extension at 72 °C for two minutes. The resulting PCR products were purified using the Qiagen PCR Purification kit and sequenced using an ABI PRISM 3730 DNA Analyser (DBS genomics Durham University).

### 3.3.4 Genetic diversity and structure

Mitochondrial DNA Sequences were aligned with a Geneious alignment in Geneious vR6 (Biomatters). The program DNAsp 10.4.9 (Rozas *et al.* 2003) was then used to calculate mtDNA polymorphism estimated as haplotypic diversity (Nei & Tajima 1981) and nucleotide diversity (Nei 1987). Mitochondrial DNA haplotypes were compared with published sequences of *L. fluviatilis* and *L. planeri* retrieved from GenBank, representing 19 additional localities throughout Europe (Espanhol *et al.* 2007; Mateus *et al.* 2011). To determine the pair-wise level of genetic differentiation between populations, F-statistics (Weir & Cockerham 1984) were calculated for mtDNA using ARLEQUIN v 3.5 (Excoffier & Lischer 2010). Significance was tested using 1000 permutations. Two tests of selective neutrality were performed in ARLEQUIN: Tajima's D (Tajima 1989) and Fu's *Fs* test (Fu 1997). These tests determine whether sequences are evolving randomly, as expected under neutral theory, or if they are affected by alternative mechanisms such as selection, gene flow, demographic expansion or decline. For both statistics, a demographic expansion produces large negative values.

Mismatch distributions, implemented using ARLEQUIN, were also used to evaluate possible events of expansion and decline (Rogers & Harpending 1992). Putative time of population expansion was estimated from the mismatch distribution using the statistic tau ($\tau$; Rogers & Harpending 1992). There is no fossil-calibrated molecular clock for lamprey mtDNA. Therefore a mutation rate was approximated using estimates calculated by Ho *et al.* (2007), which were about 50% per site per million years for the control region which can be ten times faster than the rest of the mitochondrial genome (McMillan & Palumbi 1997) giving a crude estimate of the mutation rate of 5% per site per million years for ATPase. Mutation rates of 1% and 10% per million years were also used to illustrate the effect that the rate of divergence will have in the expansion times. Population expansion times were

then estimated assuming a constant molecular clock using the tool http://www.uni-graz.at/zoowww/mismatchcalc/index.php developed by Schenekar and Weiss (2011). This allows the estimation of the time since expansion by using the formula $t=\tau/2u$ where $\tau$ is a unit of mutational time and u is the cumulative substitution rate per generation across the DNA fragment under study. The relationship between European lampreys and the populations in the British Isles was investigated using a median joining network (MJN) constructed using NETWORK 3.1.1.1 (Bandelt *et al.* 1999).

## 3.4 Results

The ATPase 6/8 gene, across 829 bp, was sequenced and haplotypes determined for 108 lampreys (including both *L. fluviatilis* and *L. planeri*) from six sampling sites (Figure 3.2). A total of 16 haplotypes defined by 14 polymorphic sites were found (Table 3.2). The highest number of haplotypes encountered was in the *L. fluviatilis* population from the River Scheldt in Belgium, which had 10 haplotypes compared to the population of *L. planeri* in the River Nidd, which exhibited only one haplotype. Nucleotide diversity was low overall 0.00082 (+/- 0.00071) and ranged from 0.00 - 0.7105 (+/- 0.1135) in the Nidd (Lp) and Scheldt (Lf) populations respectively. Overall haplotype diversity was also low 0.064762 ranging from 0.00 - 1.17895 in the Nidd (Lp) and Scheldt (Lf) populations respectively, again outlining the contrast between these two populations. Both Tajima's *D* (-2.26532, $P < 0.0001$) and Fu's *F* (-17.18160, $P < 0.0001$) were highly significant, and their large negative values are indicative of an excess number of alleles (i.e. more polymorphisms than would be expected under neutrality) as would be expected from a recent population expansion which could be due to events such as recovery after a bottleneck, or strong natural selection for a new trait (selective sweep) or to retain an existing trait (purifying

selection) (Table 3.2). In this case, this most likely caused by a recent population expansion which is consistent with the results from the other analyses carried out (Figure 3.3 and 3.4).

**Table 3.2** Diversity indices for mtDNA ATPase gene across six populations. Lp signifies *Lampetra planeri,* Lf is *Lampetra fluviatilis* and Lf Res is a freshwater-resident population of *L. fluviatilis*.

| Pop. | *N* | *H* | *π* | *h* | *D* | *Dp* | *F's* | *F'sp* |
|---|---|---|---|---|---|---|---|---|
| **Bann (Lf Res)** | 20 | 3 | 0.1947 +/- 0.1145 | 0.3 | -1.51284 | 0.049 | -1.14276 | 0.049 |
| **Dee (Lf)** | 16 | 4 | 0.3500 +/- 0.1478 | 0.5 | -1.83088 | 0.014 | -1.79042 | 0.02 |
| **Nidd (Lp)** | 18 | 1 | 0 | 0 | 0 | 1 | 0 | N.A. |
| **Scheldt (Lf)** | 20 | 10 | 0.7105 +/- 0.1135 | 1.17895 | -2.0343 | 0.002 | -7.58393 | 0 |
| **Nidd (Lf)** | 17 | 2 | 0.1176 +/- 0.1012 | 0.11765 | -1.16387 | 0.154 | -0.74844 | 0.1 |
| **Dee (Lp)** | 17 | 2 | 0.2206 +/- 0.1208 | 0.22059 | -0.49134 | 0.262 | 0.03529 | 0.242 |
| **All** | 108 | 16 | 0.00082 +/- 0.00071 | 0.064762 | -2.26532 | 0.00010 | -17.18160 | 0 |

Where *N*= sample size, *H* = number of haplotypes*, π* = nucleotide diversity, *h* = haplotype diversity, *D* = Tajima's *D*, *Dp* = Tajima's *D p*-value, *F's* = Fu's *F*, F'sp = Fu's *F p*-value.

Mismatch analysis is one method used to estimate population divergence (Slatkin & Hudson 1991; Rogers & Harpending 1992). Populations that have experienced a sudden or exponential growth or decline produce a smooth, uni-modal wave in the distribution of pairwise sequence differences (the mismatch distribution) corresponding to that event, whereby stable populations produce more steadily sloped (non-wave-like) distributions. For a uni-modal mismatch distribution, the mode is at the value of tau (τ), a moment estimator, which represents a unit of mutational time. Therefore, the time since population expansion (t) can be calculated by $t=\tau/2u$, where *u* is the cumulative (across the sequence) probability of substitution. Here, the mismatch distribution (Figure 3.3) shows evidence of expansion for all populations of *L. planeri* and *L. fluviatilis* combined. Using the value of *tau,* which was

0.673, an expansion time of 16,263 years ago was calculated using the mutation rate of 5% per million years. Using a mutation rate of 1% and 10% respectively, expansion times of 81,182 and 8,118 years ago were also calculated as outlined in the methods section.

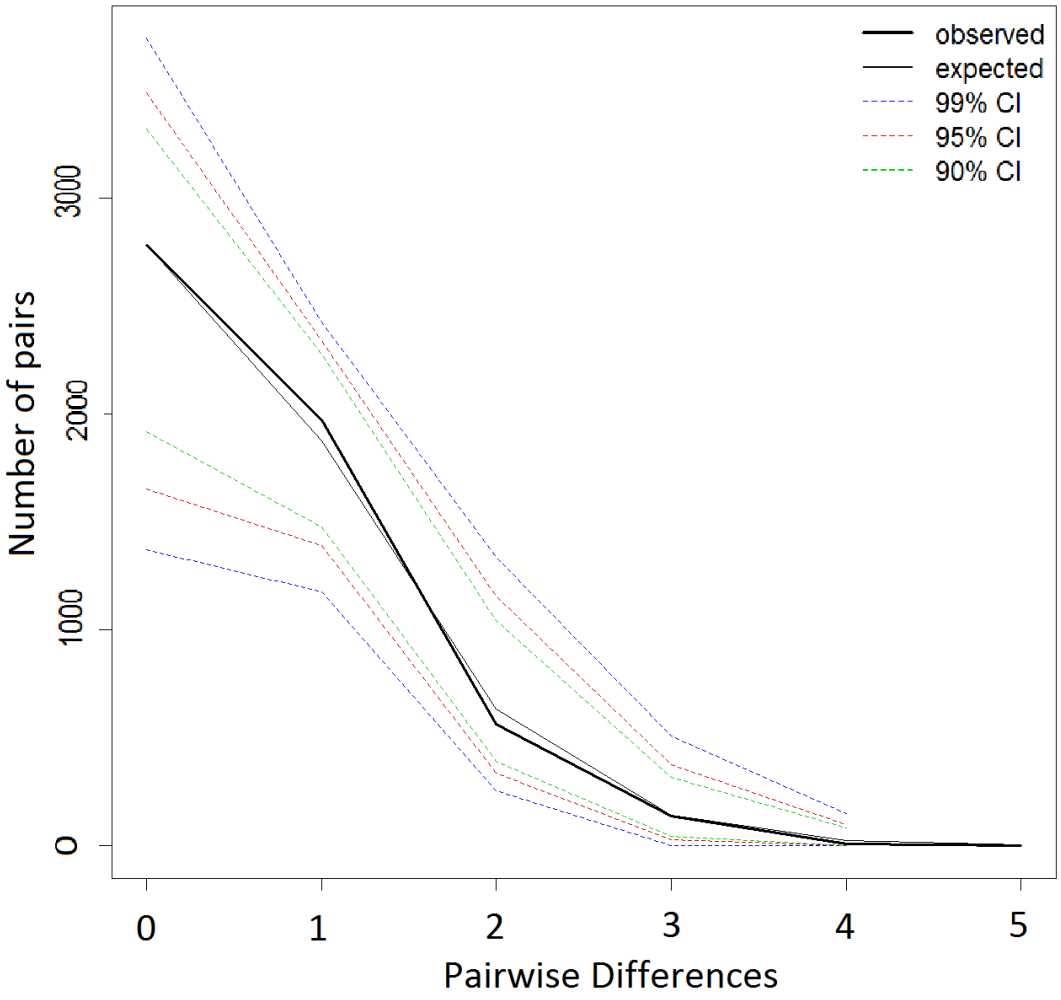



**Figure 3.3** Mismatch distribution (demographic expansion) with *Tau* 0.673, showing an expansion pattern for six populations of *Lampetra fluviatilis* and *Lampetra planeri* presented in Table 3.1.

The haplotype distribution (Table 3.2) and median joining network with distribution map (Figure 3.4) showed few differences between species or sampling locations but a clear 'star-shaped' pattern suggesting expansion. No private haplotypes or species-specific lineages were encountered. The most common haplotype (H1) was found in all populations (*L. planeri, L. fluviatilis,* and the freshwater-resident *L. fluviatilis* population in the Bann) excluding the Nidd (Lp) population. Haplotype 6 was the only haplotype included in the Nidd (Lp) population and is likely to be due to a founding event with a small number of individuals.

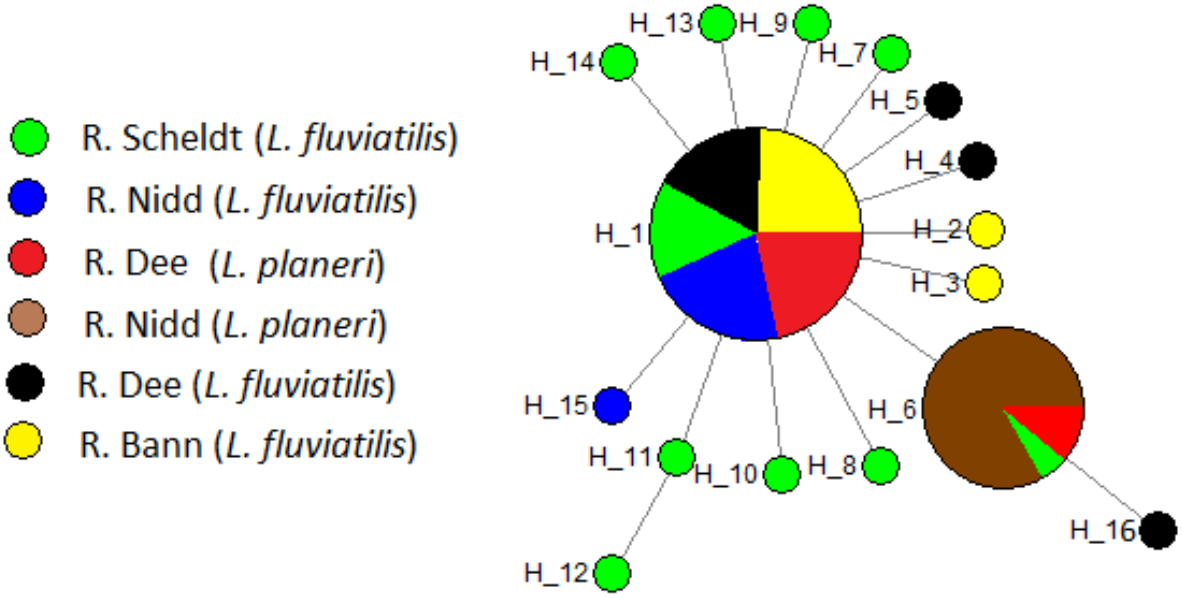



**Figure 3.4** Median-joining Network showing 16 haplotypes found from 108 samples consisting of either *Lampetra planeri*, or *Lampetra fluviatilis* from six sampling locations. Details of the sample locations are given in Figure 3.2. Note that the R. Bann is a freshwater resident *Lampetra fluviatilis* population.

$F_{ST}$ values between sites ranged from -0.01955 (Dee Lf and Dee Lp) to 0.94093 (Nidd Lf and Nidd Lp) with the lowest differentiation occurring between the Lf and Lp populations within the River Dee showing that differentiation was not species related. The highest differentiation occurred between the Nidd Lf and Lp populations, which is likely due to a founding event in the Nidd Lp population fixing a single haplotype there. The only $F_{ST}$ values that were statistically significant were those associated with the Nidd *L. planeri* population ($P < 0.0001$; Figure 3.5).

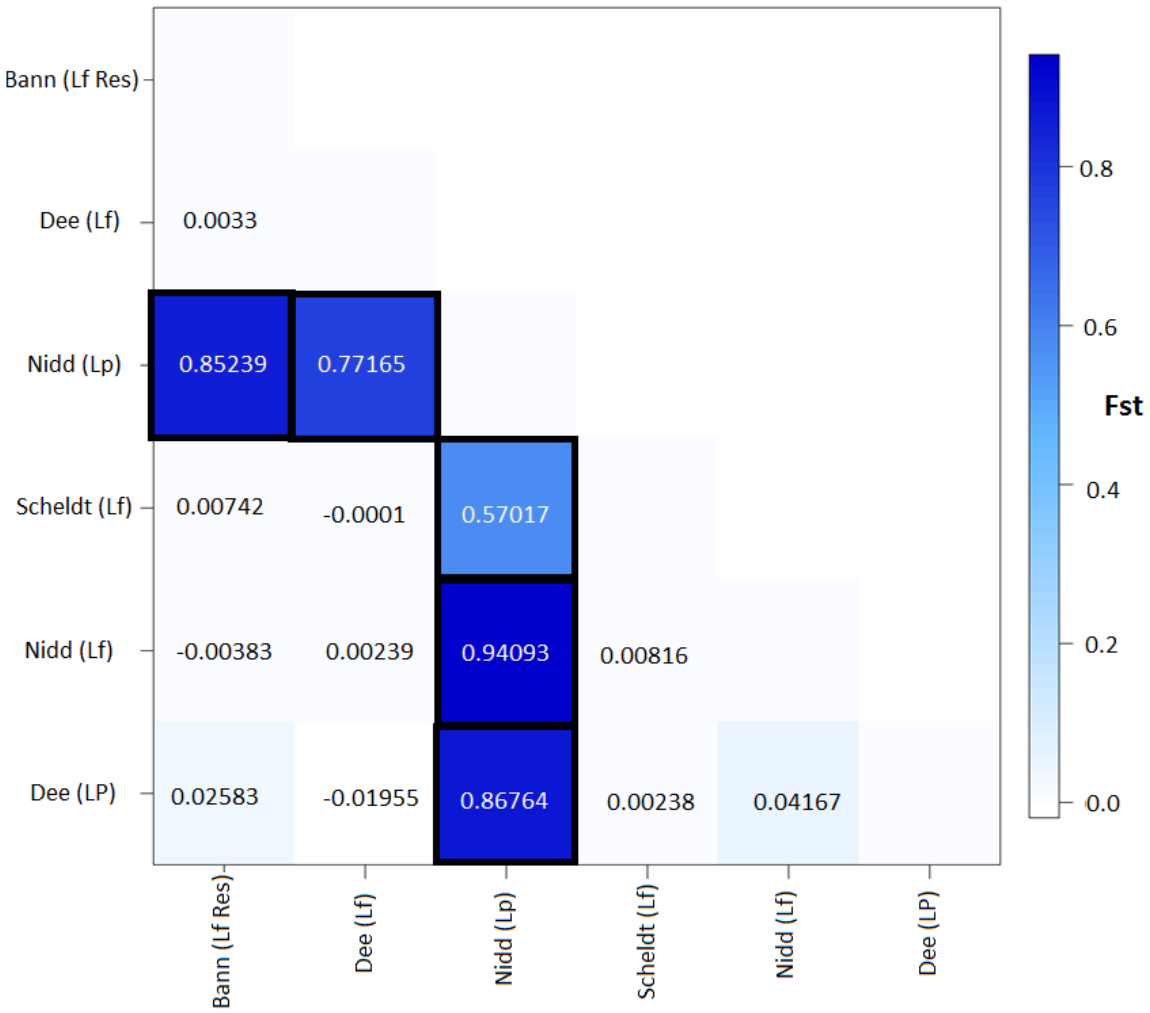



**Figure 3.5** Matrix of pairwise $F_{ST}$ values for mtDNA analysis of six populations of *Lampetra*. Abbreviations: Lf =*Lampetra fluviatilis*, Lp = *Lampetra planeri,* and Lf Res= freshwater-resident population of *L. fluviatilis*. Significant $F_{ST}$ values (i.e. all $F_{ST}$ values associated with Nidd (Lp)) are highlighted with a thick black border ($P < 0.0001$).

A network showing the European haplotype distribution, incorporating data from Espanhol *et al.* (2007) and Mateus *et al.* (2011), revealed 46 haplotypes with populations from the Iberian peninsula being further removed from the majority of other samples (Figure 3.6). The samples from northern Europe are mostly contained within one group along with some samples from the Iberian Peninsula. Identified lineages were concordant with those reported by Mateus *et al.* (2011), and as observed by Espanhol *et al.* (2007). No groupings were species specific. Clades I, II and III are composed of adults of *L. planeri* and larvae of unknown specific status, while clade IV includes both anadromous *L. fluviatilis* and freshwater-resident *L. planeri* adults and larvae.

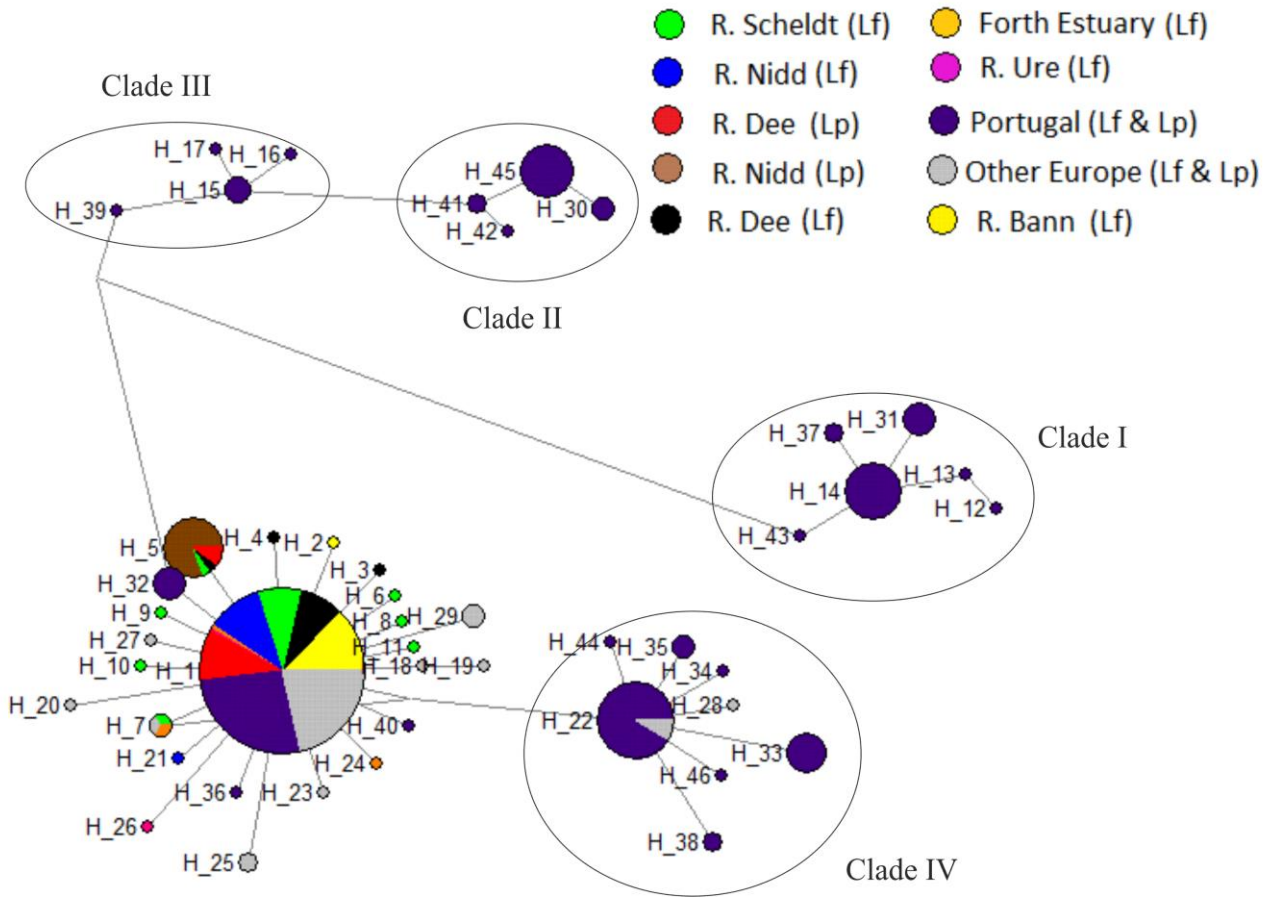



**Figure 3.6** Median joining network showing 43 haplotypes comprising of both *Lampetra fluviatilis* (Lf) and *Lampetra planeri* (Lp). Circled groups show correspondence with clades identified in Mateus *et al.* (2011). Clades I-III consist of resident *L. planeri* with restricted distribution and Clade IV contains both resident Lp and anadromous Lf with a wider distribution along with haplotypes identified in Espanhol *et al.* (2007) from France, Sweden and Germany( Lp and Lf H22) and France (Lp H28).

## 3.5 Discussion

The ATPase gene in the mitochondrial genome of *L. fluviatilis* and *L. planeri* failed to show any differentiation between the two species. This is consistent with previous studies using mtDNA which have also found that neither species is reciprocally monophyletic for mtDNA (Espanhol *et al.* 2007; Mateus *et al.* 2011). This is suggestive of either ongoing gene flow or, alternatively, incomplete sorting of ancestral polymorphisms. This is supported by the network analyses and neutrality tests, all of which are consistent with an expansion event (Figures 3.3, 3.4, 3.6 & Table 3.2). The lack of geographical structuring among haplotypes from northern Europe and the British Isles (Figure 3.4) suggests small founding populations and subsequent expansion. Other studies have shown that in central and northern Europe, lamprey populations (both *L. fluviatilis* and *L. planeri*) exhibit low nucleotide diversity and little phylogeographic structure, while in southern Europe, particularly the Iberian Peninsula, populations exhibit far higher nucleotide diversities and significant phylogeographic structuring (Pereira *et al.* 2010).

It has been generally accepted that lamprey paired-species are closely related, with the non-parasitic species having evolved from a similar form to that of the extant parasitic anadromous lamprey (Zanandrea 1959; Hardisty 1986a; Youson & Sower 2001). It is still debated however, whether there have been multiple, independent divergences of non-parasitic freshwater-resident lampreys from anadromous lampreys, exhibiting a repeated loss of anadromous ability (i.e. convergent evolution). The geographical distribution of the non-parasitic species is typically contained within the range of the parasitic form (Hardisty 1986a) as is the case with *L. fluviatilis* and *L. planeri,* which have broadly overlapping geographical ranges from northern Europe to the western Mediterranean (Chapter 1, Figure 1.2). *Lampetra planeri* does not generally occur outside

the extant range of *L. fluviatilis*, except for some isolated occurrences in the headwaters of the Danube and Volga river systems, and in some Portuguese streams (Hardisty 1986b; Almaça & Collares-Pereira 1988; Almaça & Cortes 1991; Pereira *et al.* 2010). These isolated populations of *L. planeri*, however, are likely to be the result of earlier colonisations by ancestral anadromous forms that have since been cut off by either glacial cycling, or anthropogenic barriers. Once this isolation has occurred it seems logical that these populations will consequently diverge as a result of genetic drift and adaptation to a purely freshwater environment, perhaps without access to host species.

Espanhol *et al.* (2007) found evidence to support the theory of multiple independent divergences of *L. planeri* in their analysis of mtDNA. Phylogeographical patterns across *L. fluviatilis* and *L. planeri* suggested that *L. planeri* may have indeed originated within at least two evolutionary lineages, which may have been the result of independent divergence events from *L. fluviatilis* with the repeated loss of anadromy. Pereira *et al.* (2010) have since found several populations of *L. planeri* in Portuguese streams, which are isolated among themselves and also from the anadromous lamprey population. These populations proved to be entirely composed of private haplotypes, a finding that supports a scenario in which a significant amount of time has passed to establish an independent evolutionary history for these populations. This was substantiated by the recent discovery of three new cryptic species (*Lampetra alavariensis, Lampetra auremensis* and *Lampetra lusitanica*) of non-parasitic lampreys which have diverged from *Lampetra planeri* in the Iberian Peninsula (which includes the same populations identified as having private haplotypes in the latter study by Pereira *et al.* 2010) as revealed by mtDNA markers (Mateus *et al.* 2013a). In this region, non-parasitic lampreys are confined to small, isolated river basins and have evolved in allopatry giving rise to separate

evolutionary lineages, each having smaller geographic ranges than *L. planeri*, and consequently, greater vulnerability to extinction.

In the case of non-parasitic populations existing in sympatry with anadromous lampreys, an isolating mechanism would need to exist to facilitate the divergence of *L. planeri*. If these multiple divergence events were very recent however, the newly formed species may have not yet achieved reciprocal monophyly *via* genetic drift and lineage sorting (Neigel & Avise 1986). This 'parallel speciation' essentially means that there would have to be repeated independent evolution of a reproductive isolating mechanism (Schluter & Nagel 1995). Parallel speciation has been suggested for many examples of species-pairs; the three-spined stickleback (*Gasterosteus aculeatus*) (McKinnon & Rundle 2002), sockeye salmon (*Oncorhynchus nerka*) (Taylor *et al.* 1996), and brown trout (*Salmo trutta*) (Bernatchez *et al.* 1992). If non-parasitic *L. planeri* have multiple origins, both in space and time, it is conceivable that speciation might still be in progress in some populations where both forms are not yet reproductively isolated (Espanhol *et al.* 2007).

In the past, lamprey taxonomy has primarily relied on the biological species concept (Mayr 1942), in which reproductive isolation is the central principle. If this is the case, then differences in body size at sexual maturity between these divergent life history types could act to prevent gene flow between them in the form of assortative mating or mechanical incompatibility. *Lampetra fluviatilis* and *L. planeri* exhibit marked differences in mature adult size, *L. fluviatilis* with an average length of about 30 cm and *L. planeri* averaging about 15 cm (Maitland 2003). Lampreys have been shown to generally choose mates of similar size and their fertilisation success decreases with increasing difference in body size (Hardisty & Potter 1971b; Beamish & Neville 1992). Deviation from a 1:1 body length ratio appears to reduce fertilisation success both within and between

species (Malmqvist 1983). Reproductive success in this study was examined by introducing single pairs of various combinations of body lengths into aquaria and the appearance of eggs (that began to cleave within a day) was considered to be a successful fertilisation. It was found that little to no successful spawning was found where body size difference between mates was greater than 25% (although it is notable that a small proportion of eggs between mismatched pairs did still result in fertilisation; Malmqvist 1983).

However, a recent study has shown that pre-zygotic barriers to gene flow in the form of strong assortative mating do not occur between sympatric populations of *L. fluviatilis*, *L. planeri* and freshwater-resident *L. fluviatilis* in Loch Lomond, Scotland (Hume 2013). The latter study was conducted in an artificial stream where trials were carried out by placing a single female and three males (one from each of the aforementioned populations) into a blocked-off stream section. Heterotypic mate selection was found to be a common occurrence, demonstrating that differences in adult body size relative to species/ecotype did not eliminate heterotypic individuals as potential mates and therefore assortative mating did not occur. Subsequently, alternative mating tactics, such as 'sneaker males' have also been shown to exist between parasitic and non-parasitic forms suggesting that significant levels of gene flow between putative lamprey species could still exist, despite large body size discrepancies (Hume *et al.* 2013c). *In vitro* hybridisation between *L. fluviatilis* and *L. planeri* has resulted in a high proportion of embryos capable of attaining the burrowing pro-larval stage, indicating no post-zygotic barriers to gene flow between these species (Hume *et al.* 2013b). Therefore, a combination of these 'sneaker male' tactics coupled with communal spawning, which is now known to occur with European *Lampetra* spp. (Lasne *et al.* 2010), could result in contemporary gene flow between *L. planeri* and *L. fluviatilis*.

Hardisty (1986a) suggests that non-anadromous forms might be expected to arise from polymorphic species. Alternative forms of *L. fluviatilis* exhibiting a smaller body size (usually an intermediate size between anadromous *L fluviatilis* and *L. planeri*) exist in Finland (Tuunainen *et al.* 1980), Lake Ladoga in the Baltic (Hubbs & Potter 1971) and Loch Lomond in Scotland (Maitland *et al.* 1994). These populations exhibit reduced body sizes (*c.* 20 cm total length) and lower fecundity compared to anadromous forms, and do not migrate to estuaries or the sea to feed. It is therefore possible that these populations could be some kind of morphologically intermediary form. Although the body size and the migratory behaviour of these freshwater parasitic *L. fluviatilis* populations are intermediate to the more extreme patterns of river and brook lamprey, they are not hybrids (Hardisty 1986b).

Some species of lamprey contain populations that exhibit atypical foraging strategies, which are known as 'praecox'. In *L. fluviatilis*, praecox variants are smaller in length than typical *L. fluviatilis* and (are presumed to) spend a reduced period of time feeding in marine or estuarine environments. A praecox population of *L. fluviatilis* is said to exist in the River Severn (England) which is estimated to spend 12 months feeding as opposed to larger bodied *L. fluviatilis* which are estimated to feed in the estuary for 18 months (Abou-Seedo & Potter 1979). Other populations of *L. fluviatilis* that feed exclusively in large bodies of freshwater, such as the populations at Loch Lomond (Scotland) and Lough Neagh (N. Ireland), are sometimes considered to be praecox, as these populations can be smaller in size at the conclusion of their feeding period (Tuunainen *et al.* 1980; Maitland *et al.* 1994; Adams *et al.* 2008; Inger *et al.* 2010). Individuals from these populations, however, can also exhibit the typical size for *L. fluviatilis* (Goodwin et al. 2006). Estimates of time spent feeding in marine/estuarine environments by lampreys seems to be primarily based on average sizes and seasonal catches, which are

arguably not the most reliable method. However, there are few studies that have reliably quantified the time spent feeding by these populations (by either mark-recapture or aging via statoliths).

Although feeding-type (parasitic *vs.* non-parasitic) has generally been used to taxonomically distinguish lamprey paired-species, there is some evidence for plasticity of feeding type. This occurs in the form of typically non-parasitic lampreys being capable of facultative parasitism, whilst some parasitic lampreys seem to be able to mature without feeding as adults (Renaud *et al.* 2009). For example, adult 'giant' American brook lampreys (*Lethenteron appendix* reported as *Lampetra lamottei*) have been found which are nearly twice as long, and almost six times as heavy, as normal American brook lampreys (Manion & Purvis 1971; Vladykov & Kott 1980; Cochran 2008). The parasitic counterpart of the American brook lamprey, the Arctic lamprey (*Lethenteron camtschaticum*) does not geographically overlap (Renaud *et al.* 2009), and due to the extremely low likelihood that these individuals attained this large size as filter feeders, Manion & Purvis (1971) argue that this indicates these individuals are facultative parasites. Although there is no direct evidence of feeding, this species maintains a lot of the structures required for parasitic life, including sharp teeth and glands that produce anticoagulating secretions.

Since non-parasitic lampreys tend to metamorphose at older ages and larger sizes than their paired parasitic species, it has been suggested that non-parasitism has evolved as a result of the change in timing of metamorphosis relative to the timing of sexual maturation i.e. heterochrony (a shift in the relative timing of developmental events) (Hardisty 2006). Non-parasitic lampreys have a longer larval phase than parasitic

lampreys and tend to metamorphose at older ages and larger size (Figure 3.7). However, at the initiation of metamorphosis, although the gonads are at roughly the same state of development in parasitic and non-parasitic lamprey, the maturation proceeds more rapidly during and after metamorphosis in non-parasitic species. This would result in a trade-off for non-parasitic lampreys between reduced fecundity, due to their smaller size at maturation, and the decreased risk of mortality associated with a resident life history strategy (Renaud *et al.* 2009). However, if this was a relatively plastic process, rather than a fixed developmental process, one would expect a far greater proportion of males (due to low gamete cost) to mature without going to sea (e.g. equivalent to the precocious male parr in salmon; Thorpe 1975; Saunders *et al.* 1982). The fact that this does not appear to occur is suggestive that this process may be a fixed trait rather than a strongly plastic phenotype.

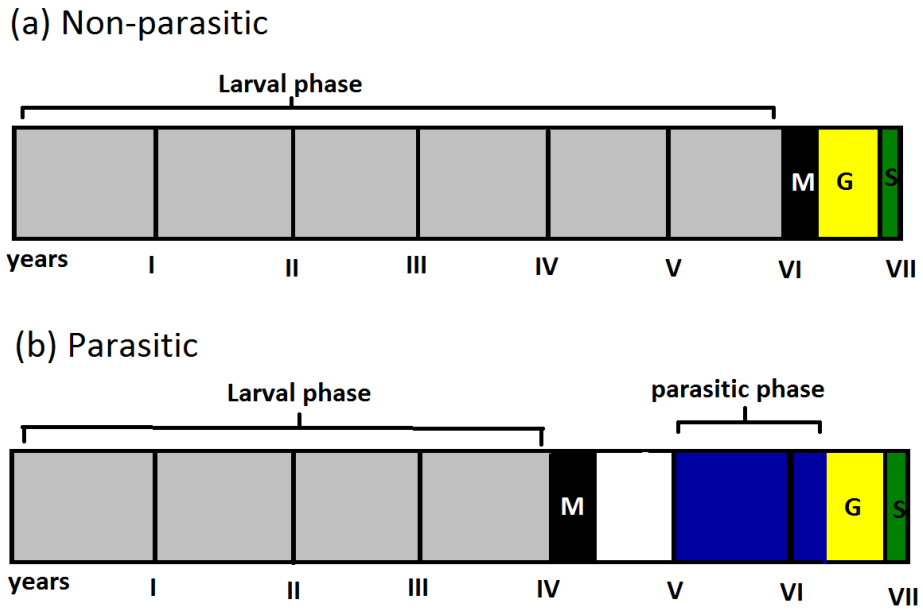



**Figure 3.7** Schematic diagram showing proposed heterochronic shift in the timing of metamorphosis relative to the timing of sexual maturation without changes to the overall lifespan in (a) non-parasitic *Lampetra planeri* and (b) parasitic anadromous *Lampetra fluviatilis*. Note that the length of the larval phase in non-parasitic lampreys is variable and may not necessarily be extended by just one year. The white section in panel (b) shows where variability may exist in timing of larval phase and metamorphosis of non-parasitic lampreys. Abbreviations are as follows: M= metamorphosis, G = gonad maturation, S = spawning. Adapted from Hardisty (2006).

Relatively little is known about the possible mechanism and change in the relative timing of sexual maturation and metamorphosis in lampreys. Many studies have explored the endocrine events that control lamprey reproduction (Sower 2003; Bryan *et al.* 2008; Freamat & Sower 2013), as well as triggers responsible for metamorphosis (Youson 1980; Youson & Sower 2001), however no studies compare paired-species, which is problematic due to larvae being indistinguishable. In each lamprey species-pair, it is not yet known to what extent morphological and ecological differences of each pair are associated with distinct gene pools, or whether an environmental trigger plays a role in the divergence of feeding types. The point at which speciation is 'complete' is vague and varies with the species concept being applied and the stringency of its application

(Hendry *et al.* 2009). Speciation does not tend to happen instantly, but usually involves a somewhat gradual accumulation of genetic differences and can be viewed as a continuum (Hendry *et al.* 2000; Schluter 2000; Rundle & Nosil 2005; Berner *et al.* 2009; Nosil *et al.* 2009). Therefore, the divergence time of non-parasitic lampreys from their anadromous ancestors is the key to a more resolved answer as to where non-parasitic lampreys stand on the speciation continuum.

This study estimates the expansion time of *L. planeri* and *L. fluviatilis* populations in the British Isles and northern Europe as 16,236 years ago using *tau* and a mutation rate of 5%/ MY (81,182 or 8,118 years ago if the mutation rate was 1% or 10%, respectively) which roughly coincides with the last glacial maximum (19 - 26,000 year ago; Clark *et al.* 2009). The Pleistocene (2.588 million (± 5,000) to 11,700 years ago) was notable for massive climatic fluctuations and much of Europe was covered by glacial-interglacial cycles (Hays *et al.* 1976; Webb & Bartlein 1992). These massive climatic and environmental changes significantly influenced the distribution and genetic diversity of plants and animals (Hofreiter & Stewart 2009). The classical 'refugium theory' proposes that temperate species survived the glacial maxima in southern refugia and colonised northern latitudes during interglacial periods (Taberlet *et al.* 1998; Hewitt 2000). The results shown here, coupled with the latitude of the Iberian Peninsula, suggests that this may have acted as an important refugium in Europe during the Pleistocene glaciations, intermittently acting as a point of dispersal for post-glacial expansion (Espanhol *et al.* 2007; Mateus *et al.* 2012; Mateus *et al.* 2013b).

The way organisms respond to climatic oscillations like the glacial cycles of the Pleistocene is dependent on a wide range of factors, such as life-history, behaviour,

thermal preferences and physiology, and specific habitat requirements (Pereira & Almada 2013). The fact that genetically distinct non-migratory *Lampetra* populations are found in many Portuguese rivers (Pereira *et al.* 2010; Mateus *et al.* 2011; Mateus *et al.* 2013a), suggests lampreys were once more abundant and widespread in Iberia. The higher levels of divergence shown in the median joining network, which included Portuguese lampreys (Figure 3.6), compared to other populations examined across Europe, also suggests that sufficient time may have passed to establish a complex of incipient resident species (that exist as isolated populations).

These findings are in agreement with a number of phylogeographical studies (Alexandrino *et al.* 2002; Gante *et al.* 2009; Mateus *et al.* 2011) that also provide evidence of considerable genetic divergence within the Iberian Peninsula refugia. Many species have been shown to display a strong population sub-structure within Iberia and are actually composed of isolated populations in distinct Iberian sub-refugia as a consequence of extended periods of isolation throughout the ice ages (Gómez & Lunt 2006). *Lampetra fluviatilis* is presumed to be extinct in Spain, which is reportedly a consequence of the construction of several dams in the Tagus river and was last recorded there in 1974 (Doadrio 2001). It therefore seems likely that *L. fluviatilis* in the Iberian Peninsula is at the extreme south of its range, being a relict population (Pereira & Almada 2013). *Lampetra planeri*, however, shows a wider distribution in the Iberian Peninsula and its presence has been confirmed in several river basins in Portugal (Espanhol *et al.* 2007; Mateus *et al.* 2011).

Therefore, it seems conceivable that during inter-glacials, while migratory *Lampetra* were expanding northwards, populations at lower latitudes would tend to abandon anadromy and eventually become restricted to freshwater. A repeated pattern of freshwater

residency due to glacial-interglacial cycling could also lead to reduced gene flow with sea-migrating populations and the eventual loss of anadromous ability in some populations (Mateus *et al.* 2011). This is consistent with the findings of a recent study utilising restriction site associated DNA sequencing (RAD seq.) that identified strong genetic differentiation between *L. fluviatilis* and *L. planeri* in the Iberian Peninsula with numerous fixed and diagnostic single nucleotide polymorphisms (SNPs) between the two putative species (Mateus *et al.* 2013b). The median joining network in Figure 3.6 shows that for the available samples, only clade IV shares a haplotype with the lineage representing the northern expansion, suggesting a possible link between these lineages (with clade IV providing the ancestor of the anadromous group that founded the postglacial population in northern Europe). With expansion into previously unoccupied territory, it is expected that genetic diversity should decrease from the south to the north (Hewitt 1996). This is consistent with the findings here.

It seems apparent that an initial phase for the divergence of this species pair involved a post-glacial expansion of anadromous *L. fluviatilis* from southern refugia and then possibly the subsequent establishment of multiple philopatric (in the sense of habitat fidelity) freshwater-resident *L. planeri* populations. In contrast to the relatively stable environment of the southern geographic range (e.g. in Portugal), the northern populations were apparently established after the last glacial maximum (LGM), and the process of differentiation is likely ongoing. Often little genetic divergence is found between ecologically and morphologically differentiated populations or species (Orr & Smith 1998). However, using neutral markers that are unaffected by selective pressures, allows the fine scale detection of changes in allele frequencies within and between populations (Frankham *et al.* 2002). Therefore further exploration using microsatellite

loci (chapter 4) could provide greater insight into the evolution of diversity of the European lamprey species pair *Lampetra fluviatilis* and *L. planeri*.

NOW look at Ammocoetes there, reclining in the mud,
Preparing thyroid extract to secure his tiny food;
If just a touch of sunshine should make his gonads grow
The lancelet's claim to ancestry would get nasty a blow.
—*W. Garstang*, The Axolotl and the Ammocoete

"The Creator told the people that the eels (lampreys) would always return as long as the people took care of them, but if the people failed to take care of them, they would disappear."
—*Ron Suppah,* Vice Chair, Warm Springs Tribes.

# Chapter 4 Contrasting population genetic structure among freshwater-resident and anadromous lampreys: differential habitat fidelity with anthropogenic barriers to movement.

## 4.1 Introduction

Although loss of migratory ability remains a complex process within an evolutionary trajectory, there is evidence to suggest that this phenomenon might act as a driver and initiator for adaptive changes within populations (Bell & Andrews 1997; Winker 2000; Räsänen & Hendry 2008; Langerhans & Riesch 2013). Differences in life history traits between resident and migrant individuals can be thought of as adaptive behaviours that act to optimise growth, survival rate, fecundity and egg quality. This is reflected in the fitness outcomes of both life history strategies, with residency favoured when the cost of migration exceeds it's benefits, particularly in terms of growth potential and mortality risk before reproduction (Fryxell & Sinclair 1988; Bell & Andrews 1997; Dingle 2006; Brönmark *et al.* 2008; Shaw & Couzin 2013).

Anadromy, which involves reproduction in freshwater and the majority of growth in the marine environment, is a distinctive migratory trait that is recognised in 18 fish families and 120 species (McDowall 1997; Chapman *et al.* 2012). Anadromy inherently offers an opportunity to colonise previously unexploited freshwater environments, and the shift from an anadromous to a wholly freshwater life history has occurred repeatedly in many families of fishes (Petromyzontidae, Salmonidae, and Galaxiidae; Potter 1980; Taylor *et al.* 1996; Waters & Wallis 2001). Glaciation or deglaciation events may have supported the divergence of wholly-freshwater forms (See Chapter 3, Section 3.1) by either blocking migratory routes and preventing anadromy or upon deglaciation, making

available new habitat and food resources that are inaccessible through freshwater but easily reached by anadromous fish (Bell & Andrews 1997; Lee & Bell 1999).

The extent to which anadromy is obligatory varies among species. Many populations of anadromous fishes contain a component that does not migrate to sea and instead remains in freshwater where they mature and spawn, in some cases moving little, but in others migrating between distinct freshwater habitats (potamodromy), often reproducing with their anadromous conspecifics (McDowall 2001). 'Partial migration' is the term coined for this resident - migratory dimorphism in populations and is widespread, with many examples of partially migratory mammals, invertebrates, birds (Lundberg 1988; Jahn *et al.* 2010; Chapman *et al.* 2011; Chapman *et al.* 2012) and fishes (Tsukamoto & Arai 2001; Olsson & Greenberg 2004; Brodersen *et al.* 2008; Kerr *et al.* 2009). Jonsson and Jonssson (1993) have shown this to be a trend in many salmonid species (e.g. *Salmo trutta, S. salar, Salvelinus fontinalis, S. alpinus, S. malma* and *Oncorhynchus nerka*) and in anadromous systems this has been linked to alternative reproductive tactics such as smaller residents acting as 'sneakers' to achieve mating success (Chapman *et al.* 2011). The mechanisms that influence individual variation in migratory behaviour are presently contentious, with various factors seeming to act synergistically to create complex patterns within populations. Nonetheless, growth and/or body size at maturation seem to play a significant role in the probability of migration with fast growing individuals more likely to migrate and individuals that reach maturation at an earlier stage, more likely to remain 'resident' (Chapman *et al.* 2011; Acolas *et al.* 2012).

Partial migration has the potential to promote incipient speciation through both allopatric and sympatric mechanisms (Chapman *et al.* 2011). Reduced gene flow between

migrants and residents breeding in allopatry could promote differentiation by genetic drift or local adaptation (Schluter *et al.* 2001). In systems where sympatric breeding occurs, the emergence of a wholly-freshwater phenotype may influence mate choice and could potentially lead to assortative mating (Chapman *et al.* 2011). Conversely, population differentiation is limited by the large-scale dispersal capacity of migrants, resulting in a greater chance of panmixia (Hoarau *et al.* 2002; Coltman *et al.* 2007). Migratory populations that exhibit philopatry, however, can maintain discrete genetic differences between populations within species. For example, pelagic seabirds may disperse over thousands of kilometres, yet exhibit population genetic structuring within ocean basins e.g. razorbills *Alca torda* (Moum & Árnason 2001) and red-legged kittiwakes *Rissa brevirostris* (Patirana *et al.* 2002). In this way, the genetic population structure of migratory fishes may reflect the degree of dispersal within populations, which can often be attributed to differences among species' life history strategies (Goodman *et al.* 2008). For example, anadromous salmonids (*Oncorhynchus* spp.) exhibit high reproductive site fidelity, and as a result there is little gene flow among populations, which promotes localised adaptation and genetic differentiation resulting in a relatively high level of genetic differentiation among populations (Taylor 1991; Dittman & Quinn 1996).

In contrast to anadromous salmonids, anadromous lampreys generally show very low inter-population differentiation across geographically distant river systems (Almada *et al.* 2008; Goodman *et al.* 2008). Migrating anadromous adult lampreys do not exhibit reproductive site fidelity as many salmonids do, and have been shown to use pheromones released by stream dwelling larvae as cues to find suitable spawning habitats (Fine *et al.* 2004). In sea lamprey *Petromyzon marinus,* mitochondrial DNA has revealed regional panmixia which is consistent with a lack of reproductive site fidelity

(Waldman *et al.* 2008). Adult European river lamprey *Lampetra fluviatilis* are smaller in size, and tend to stay in areas nearer to estuaries and coastal shelf habitats than *P. marinus* (Maitland 2003), and despite having a higher probability of breeding in their natal river, they also do not demonstrate any no strong tendency to home (Tuunainen *et al.* 1980).

As outlined in Chapter 3 (Section 3.1), there has been much dispute over the taxonomic status of lamprey species-pairs. Non-parasitism has arisen repeatedly among lampreys (Docker 2009) and even within species (Mateus *et al.* 2012), suggesting that feeding-type is plastic and non-parasitic populations may be polyphyletic (Hubbs & Potter 1971; Docker 2009). Although previous studies have found difficulties in differentiating between lamprey paired species (Docker *et al.* 1999; Yamazaki *et al.* 2006; Espanhol *et al.* 2007; Blank *et al.* 2008; Lang *et al.* 2009), a recent study based on nuclear genomic data has found significant differentiation between sympatric European river lamprey (*L. fluviatilis*) and European brook lamprey (*Lampetra planeri*) populations in Portugal (Mateus *et al.* 2013b). The use of mitochondrial DNA loci, however, revealed a lack of differentiation between *L. fluviatilis* and *L. planeri* (Chapter 3). Considering that mtDNA (maternally inherited) generally provides lower resolution than nuclear markers (bi-parentally inherited), this chapter has utilised nuclear (microsatellite) loci to further explore the population genetics of anadromous *L. fluviatilis* and its non-parasitic derivative *L. planeri,* together with several *L. fluviatilis* populations that contain potamodromous individuals that migrate within freshwater only (Morris 1989; Maitland *et al.* 1994; Goodwin *et al.* 2006). This should reveal contemporary patterns of interactions among populations (microsatellites) and, in combination with information about historic patterns of colonisation (Chapter 3), gives a more holistic picture of the European *Lampetra* species pair.

There is also a possibility that anthropogenic barriers may be causing increased isolation of some lamprey populations and this was also investigated. In some freshwater fishes, the fragmentation of habitats by dams can promote genetic differentiation between the upstream and downstream populations resulting from the reduction of gene flow as well as the founder effect and subsequent genetic drift (Yamamoto *et al.* 2004; Palkovacs *et al.* 2008). Through analysis of molecular markers, population divergence and dispersal at local to catchment scales were examined, enabling inferences about population connectivity and evolutionary viability, which may have important applications for conservation management (Latta 2008). The assessment of biodiversity within and among populations is central to identifying and prioritising areas for monitoring, conservation management and protection by prioritising the maintenance of levels of gene flow and maximum gene diversity (Moritz & Faith 1998; Crandall *et al.* 2000). Factors that may affect on patterns of gene flow, including life history strategy and anthropogenic habitat modification such as barriers, are consequently examined within this study which ultimately enhances our understanding of these species with a view to support more effective management strategies in the future.

## 4.2 Aims

A combination of mtDNA (Chapter 3) and polymorphic microsatellite loci were used to test the hypothesis that the post-glacial expansion of anadromous *L. fluviatilis* into northern Europe prompted the establishment of multiple freshwater-resident *L. planeri* populations that subsequently became genetically differentiated. This scenario would be consistent with observations where *L. planeri* and *L. fluviatilis* populations from the same river were genetically closer to each other than populations of geographically distict *L. planeri* are to each other. This chapter also aimed to assess contemporary and long-term

gene-flow between *L. planeri* and *L. fluviatilis* populations at paired sites (i.e. populations of *L. planeri* and *L. fluviatilis* from the same river). The possibility that anthropogenic barriers are further isolating populations and consequently affecting gene flow between them has also been investigated, along with the implications for conservation management.

## 4.3 Methods

### 4.3.1 Sampling

Tissue samples were collected from a total of 543 lampreys across 18 sites (Figure 4.1, Table 4.1). Lampreys were collected from seven systems where anadromous *L. fluviatilis* and freshwater *L. planeri* occur in reasonably close proximity (i.e. seven paired sites), with one of these sites also including a freshwater-resident *L. fluviatilis* population. Two additional sites for *L. fluviatilis* are also included in the study; one from Belgium (outside the British Isles), and one from N. Ireland, which is also a freshwater- resident population of *L. fluviatilis*. In Loch Lomond (Scotland), all three 'ecotypes' (i.e. *L. planeri*, *L. fluviatilis,* and freshwater-resident *L. fluviatilis*) are sympatric, however, in all other paired sites *L. planeri* samples were obtained upstream (within the same river) of anadromous *L. fluviatilis* populations, which were usually separated by migration barriers (Table 4.2). It should also be noted that the location from which the River Swale *L. planeri* samples were obtained is not only a spawning site for *L. planeri* as *L. fluviatilis* are also known to spawn at this location.

Geographic distances were calculated between sample sites (based on the shortest possible migration route) using linear referencing tools in Quantum GIS (Lisboa). A

barrier was defined as any feature larger than 0.5 m height at base river level which spans the full width of the river. An additional barrier was added to the total when the route between a population of *L planeri* and another population involved passing through a marine environment. A database, containing information on the specific locations, dimensions, and quantity of barriers within catchments of interest, was obtained from both Natural Resources Wales and the Environment Agency and was utilised to calculate the number of barriers between sampling locations. Due to the lack of lamprey-specific fish passes within the British Isles, all barriers that contained a fish pass were also included as a barrier to migration.

**Table 4.1** Numbers collected and location of origin for all genetic samples of *Lampetra planeri* and *Lampetra fluviatilis* and by whom they were collected. Sites 1-8 are all part of the Ouse sub-catchment within the Humber, while site 9 is in the Trent sub-catchment (Figure 4.1).

| Site no. on Figure4.1 | Country | Catchment | River | Latitude | Longitude | Species | *N* | Method of collection | Collected by: |
|---|---|---|---|---|---|---|---|---|---|
| 1 | England | Humber | Nidd | 53°58'49.00"N | 1°19'5.99" W | *L. fluviatilis* | 30 | Hand net | F. Bracken |
| 2 | England | Humber | Nidd | 54° 4'38.33" N | 1°44'48.81" W | *L. planeri* | 30 | Hand net | F. Bracken |
| 3 | England | Humber | Swale | 54°21'28.20" N | 1°32'59.70" W | *L. fluviatilis* | 32 | Hand net | F. Bracken |
| 4 | England | Humber | Skeeby Beck (Swale) | 54°25'16.14" N | 1°41'14.29" W | *L. planeri* | 11 | Electro-fishing | F. Bracken |
| 5 | England | Humber | Ure | 54° 5'50.94" N | 1°23'44.63" W | *L. fluviatilis* | 30 | Hand net | F. Bracken |
| 6 | England | Humber | Burn (Ure) | 54°13'1.92" N | 1°43'27.06" W | *L. planeri* | 30 | Electro-fishing | F. Bracken |
| 7 | England | Humber | Derwent | 53°59'31.06" N | 0°54'50.49" W | *L. fluviatilis* | 30 | Hand net | F. Bracken |
| 8 | England | Humber | Rye (Derwent) | 54°14'13.29" N | 1° 2'32.93" W | *L. planeri* | 32 | Hand net | F. Bracken |
| 9 | England | Humber | Trent | 53° 8'41.27" N | 0°47'28.42" W | *L. fluviatilis* | 33 | Trap | F. Bracken & P. Bird |
| 10 | England | Wear | Wear | 54°46'49.31" N | 1°34'34.85" W | *L. fluviatilis* | 43 | Hand net | F. Bracken & M. Lucas |
| 11 | England | Wear | Bollihope Burn | 54°43'19.28" N | 1°56'37.19" W | *L. planeri* | 30 | Electro-fishing | F. Bracken & M. Lucas |
| 12 | Wales | Dee | Dee | 53°11'11.37"N | 2°53'14.43"W | *L. fluviatilis* | 33 | Trap and electro-fishing | I. Davidson, R. Cove & F. Bracken |
| 13 | Wales | Dee | Ceiriog | 52°55'35.29" N | 3° 4'58.55" W | *L. planeri* | 30 | Electro-fishing | F. Bracken |
| 14 | Scotland | Loch Lomond | Endrick Water | 56° 3'17.31"N | 4°27'16.28"W | *L. fluviatilis* (Anadromous) | 24 | Trap | J. Hume |
| 15 | Scotland | Loch Lomond | Endrick Water | 56° 3'17.31"N | 4°27'16.28"W | *L. fluviatilis* (Resident) | 31 | Trap | J. Hume |
| 16 | Scotland | Loch Lomond | Endrick Water | 56° 3'17.31"N | 4°27'16.28"W | *L. planeri* | 36 | Trap | J. Hume |
| 17 | N. Ireland | Bann | Bann | 54°45'18.69" N | 6°27'51.06" W | *L. fluviatilis* (Resident) | 25 | Trap | Clare Goodwin |
| 18 | Belgium | Scheldt | Scheldt | 51° 0'25.89" N | 3°45'7.89" E | *L. fluviatilis* | 35 | Trap | David Buysse & Johan Coeck |
| | | | | | | Total | 543 | | |

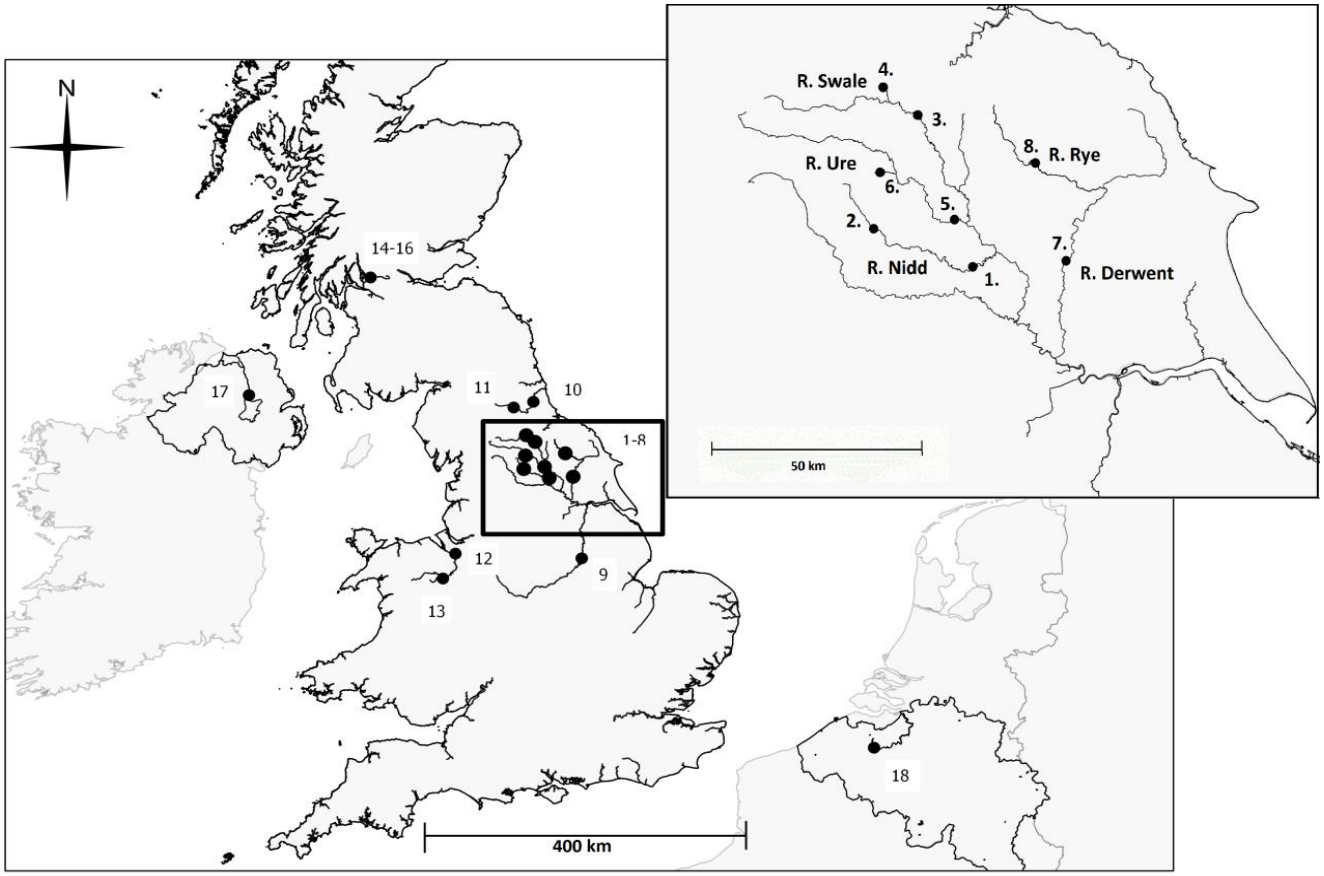



**Figure 4.1** Map showing location of sampling sites 1-18 (see Table 4.1 for detail). Inset is a detailed map of part of the Ouse sub-catchment of the Humber catchment, showing sampling locations. Excluding the River Wharfe in the Ouse sub-catchment, only rivers sampled are shown.

**Table 4.2** Number of barriers (distance km) between sampling locations. Boxes outlined with border signify paired sites. Lp = *Lampetra planeri*, Lf = *Lampetra fluviatilis*. Blue boxes represent barriers between *L. planeri* populations, red boxes signify barriers between *L. planeri* and *L. fluviatilis* populations, and green boxes represent barriers between *L. fluviatilis* populations. Geographic distances were calculated between sample sites (based on the shortest possible route) using linear referencing tools in Quantum GIS (Lisboa). A barrier was defined as any feature larger than 0.5 m height at base river level which reaches the full width of the river.

| | Derwent (Lf) | Derwent (Lp) | Nidd (Lf) | Nidd (Lp) | Ure (Lf) | Ure (Lp) | Swale (Lf) | Swale (Lp) | Wear (Lf) | Wear (Lp) | Dee (Lf) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Derwent (Lp)** | **7** (54.6) | | | | | | | | | | |
| **Nidd (Lf)** | **4** (102.6) | **11** (157.2) | | | | | | | | | |
| **Nidd (Lp)** | **14** (147.7) | **21** (202.3) | **10** (45.1) | | | | | | | | |
| **Ure (Lf)** | **4** (105.45) | **11** (160.05) | **2** (36.08) | **12** (81.8) | | | | | | | |
| **Ure (Lp)** | **11** (143.25) | **18** (197.85) | **9** (73.88) | **19** (118.98) | **7** (37.8) | | | | | | |
| **Swale (Lf)** | **6** (150.85) | **13** (205.45) | **4** (81.4) | **14** (126.5) | **2** (53.5) | **9** (91.3) | | | | | |
| **Swale (Lp)** | **7** (167.25) | **14** (221.85) | **5** (97.8) | **15** (142.9) | **3** (69.9) | **10** (107.7) | **1** (16.4) | | | | |
| **Wear (Lf)** | **3** (373.4) | **10** (428) | **3** (404.9) | **13** (450) | **3** (415.4) | **10** (453.2) | **5** (452) | **6** (468.43) | | | |
| **Wear (Lp)** | **11** (419.1) | **19** (473.7) | **13** (450.6) | **23** (495.7) | **13** (461.1) | **20** (498.9) | **15** (497.7) | **16** (514.13) | **9** (45.7) | | |
| **Dee (Lf)** | **2** (1784.6) | **9** (1839.2) | **2** (1817.1) | **12** (1862.2) | **2** (1826.6) | **9** (1864.4) | **4** (1870.46) | **5** (1886.86) | **1** (1427.7) | **11** (1473.4) | |
| **Dee (Lp)** | **7** (1845.7) | **15** (1900.3) | **7** (1878.2) | **17** (1923.3) | **7** (1887.7) | **14** (1925.5) | **9** (1931.56) | **10** (1947.96) | **6** (1488.8) | **15** (1534.5) | **4** (61.1) |

LP-LP
LP-LF
LF-LF

*Lampetra fluviatilis* and *L. planeri* samples were obtained by either hand-netting spawning adults, electric-fishing, or trapping (for the River Dee, River Bann and River Scheldt) as described in Chapter 3 (Section 3.2.1). Trapping in the River Trent (north-east England) was carried out by Paul Bird using un-baited static two-funnel traps (*c.* 1 m long, 0.30 m diameter, 12 mm mesh to first funnel, 10 mm mesh after first funnel, constructed from netting, surrounded by flexible plastic) and were fished in midstream, from one or two lines attached to a weighted rope spanning the river (Masters *et al.* 2006). Species identification and the taking of fin clips were carried out by F. Bracken on site. In the Endrick water (Scotland), adult lampreys were caught, identified and sampled (i.e. fin clips and measurements taken) by J. Hume (Hume 2013) using static, double-funnel traps that were based on the design outlined by Morris and Maitland (1987). The Endrick Water, Loch Lomond, Scotland, is a designated Special Area of Conservation (SAC) that is listed for the stream-resident *L. planeri,* as well as a population of *L. fluviatilis* containing both an anadromous and a freshwater-resident component (i.e. the potamodromous *L. fluviatilis* population) (Bond 2003).

Both *L. fluviatilis* and *L. planeri* were sampled where they were found to be locally abundant prior to their spawning period and so were, in most cases, captured in the vicinity of their spawning grounds. *Lampetra planeri* were normally captured in the upstream reaches of rivers where they were abundant and in all cases, except at the Endrick Water, Loch Lomond, were sampled upstream of the predominant *L. fluviatilis* spawning areas. Only adult and juvenile lampreys unambiguously identifiable to species were included in this study. Adult anadromous, and freshwater-resident *L. fluviatilis* (e.g. Loch Lomond; Morris 1989), as well as non-parasitic *L. planeri* can be separated using standard lamprey taxonomic characteristics (Renaud 2011). Individuals were identified and measured under anaesthesia

(MS-222, 0.1 g L-1) using a field key (Gardiner 2003) and fin clips taken from the second dorsal fin were stored in 20% DMSO saturated NaCl solution (Amos & Hoelzel 1991).

### 4.3.2 DNA extraction from tissue samples

A combination of phenol-chloroform (Sambrook *et al.* 1989) and high salt extraction (Aljanabi & Martinez 1997) methods were used to isolate DNA from tissue samples. Approximately 1/3 of the fin material was removed and finely chopped. Samples were digested at 37 °C overnight in 500 μL of digestion buffer (50 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl, 1% w/v SDS) with 0.6 mg $ml^{-1}$ proteinase K (Hoelzel & Green 1998). Both methods were followed by ethanol precipitations using sodium acetate as the source of sodium. The presence of genomic DNA was then confirmed by viewing results on 1.2% agarose gels which were run for 20 minutes alongside a 1 Kb DNA ladder. DNA was then stored in 1X TE buffer at an approximate concentration of 100-200 ng $\mu L^{-1}$ at –20°C.

### 4.3.3 Development of Microsatellites

Oligonucleotides (primers) are designed to bind to known DNA sequences (such as those flanking microsatellites) and amplify DNA of a predicted size through the Polymerase Chain Reaction (PCR). Due to their high specificity, a given pair of microsatellite primers will rarely work across broad taxonomic groups, therefore the development of new primers was needed to study the population genetics of European *Lampetra* spp. Before primers can be designed, microsatellite loci need to be isolated. This is done by the construction of a restricted DNA library and screening with microsatellite-specific probes (e.g. CA tandem repeats). Microsatellites were isolated according to the following protocol (Fischer &

Bachmann 1998), which uses the microsatellite hybrid capture technique for isolating microsatellite loci (Brown *et al.* 1995; Prochazka 1996). This technique is a variation of the methods of Kandpal *et al.* (1994), which uses microsatellite probes attached to magnetic beads to isolate pieces of DNA containing the microsatellites.

**The following provides an overview of the methodology used to develop microsatellites which is ordered in the succeeding key steps:**

1. Extraction, quantification and digestion of high molecular weight DNA
2. Gel purification and size selection
3. Ligation of linkers A-B and to size fractioned DNA
4. Amplification of fragments to create PCR-amplified library
5. Hybridisation of fragments to biotinylated probe and capture of probe using dynal streptavidin beads
6. PCR amplification of microsatellite containing fragments using linker as primer
7. P Gem-T Easy cloning of fragments: Ligation
8. Transformation of plasmid vector into bacterial (*E. coli*) cells
9. Screening colonies with PCR
10. Mini-prep to isolate plasmid and digest with Eco-RI to verify positive colonies contain inserts
11. Sequencing
12. Design primers and amplify in both *L. planeri* and *L. fluviatilis* testing for polymorphism

*Extraction, quantification and digestion of high molecular weight DNA*

The DNA was extracted using the standard phenol–chloroform protocol (outlined above). DNA was extracted from five individuals of each species (*L. fluviatilis* and *L planeri*) and was pooled to give approximately 5 μg DNA. This was then digested using the Sau3A1 (Promega) restriction enzyme (a protein produced by bacteria that cleaves DNA at specific sites) into fragments of a suitable size (usually in the range 300-600 bp).

*Gel purification and size selection*

Once the DNA was fragmented by the restriction enzyme, the digest was run on a TBE (Tris-Borate-EDTA) gel. DNA fragments between 400 and 800 bp were then gel extracted and column purified (Qiagen).

*Ligation of linkers A-B and to size fractioned DNA*

Short linkers of known DNA sequences were ligated (attached) to the ends of the restricted DNA and used to design primers that amplify target fragments in PCR reactions. Double stranded linkers were made by annealing 5 uL of each 50 uM Linker A (GCGGTACCCGGGAAGCTTGG) and Linker B (GATCCCAAGCTTCCCGGGTACCGC) primers at 68 ºC for 5 minutes together to form Linker AB. Linker AB was then ligated to the DNA fragments using T4 ligase (Promega) by incubating at 15 ºC for 5 hours.

*Amplification of fragments to create PCR-amplified library*

PCR amplification was then carried out using Linker A as the primer. PCR was performed in a G-Storm GS1 thermocycler (GeneTechnologies Ltd.) in a final volume of 30 uL

containing 21.3 uL of ddH2O, 1 uL of ligation reaction, 3 uL of buffer, 3 uL of dNTPs, 1.2 uL of primer and 0.5 uL of Taq, with a PCR profile of 96 °C for 2 min, 30 cycles of 94 °C for 40 s, 54 °C for 40 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. Excess linker was then removed from the genomic DNA using a QIAquick PCR purification kit (Qiagen).

*Hybridisation of fragments to biotinylated probe and capture of probe using dynal streptavidin beads*

Purified PCR products were denatured and incubated with 5 μg of 50 biotinylated probes (MWGBiotech AG). The microsatellite probes (dinucleotide repeat: CA) were attached to streptavidin-coated magnetic beads (G. Kisker GbmH). Probes were modified at the 3' end with 20, 30-dideoxy (Eurofins MWG), to eliminate amplification artefacts (Kobliz˘kova´ *et al.* 1998). The application of a small magnet then pulled these probes out of the solution. The microsatellite probes were attached to the beads, and any DNA fragments containing microsatellites were hybridised to these probes, therefore, the microsatellite DNA was also pulled out of solution.

*PCR amplification of microsatellite containing fragments using linker as primer*

The selected DNA was then amplified using Linker A as a primer. PCR was carried out in a final volume of 30 uL under the following conditions: 96 °C for 2 min, 30 cycles of 94 °C for 40 s, 54 °C for 40 s, 72° C for 1 min, and a final extension step at 72 °C for 10 min.

*P Gem-T Easy cloning of fragments: Ligation*

Purified PCR products were then ligated into the pGEM-T EASY plasmid vector (Promega; Figure 4.2) and cloned into *E. coli* bacteria.

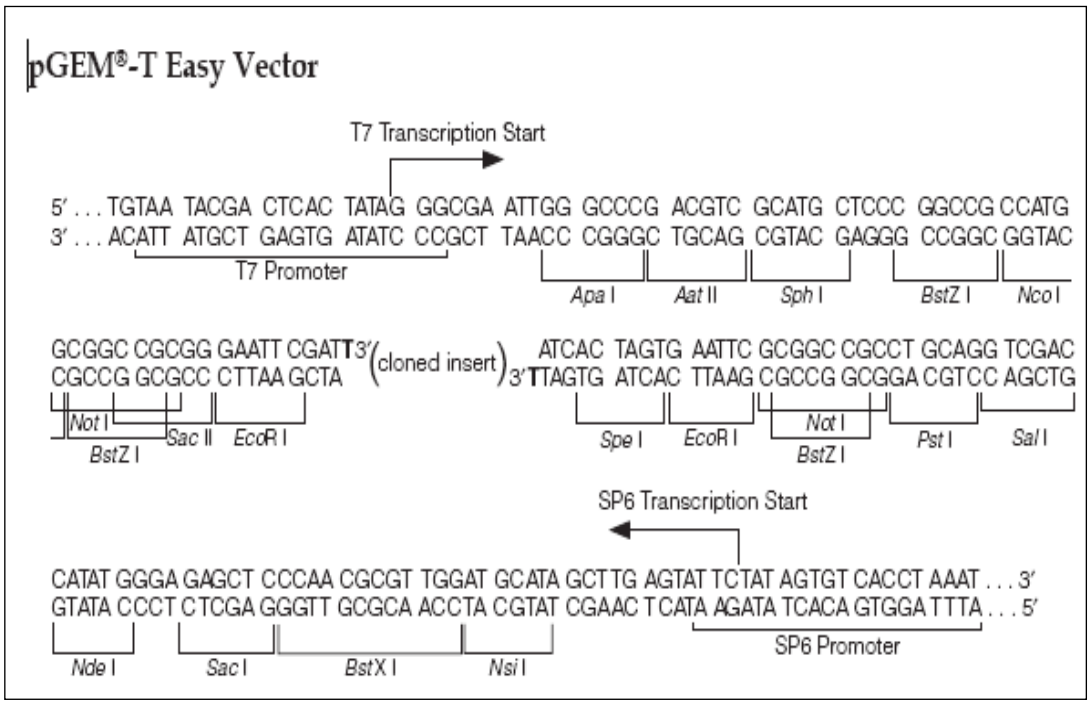



**Figure 4.2** pGEM-T EASY plasmid vector showing T7 and SP6 primer locations.

*Transformation of plasmid vector into bacterial (E. coli) cells*

*E. coli* cells (XL1 Blues) were subsequently transformed (i.e. genetically modified by the incorporation of vector + insert DNA). Transformed cultures were plated onto LB/AMP/TET/IPTG/X-GAL plates and incubated overnight at 37° C. After overnight culture of the plates, individual colonies were present that were colour coded to indicate the presence of the insert.

*Screening colonies with PCR*

Positive cultures were then amplified, using PCR primers designed based on vector sequence, to determine the size of the insert (the DNA fragment containing the microsatellite). Colony PCR was performed using pGEM-T Easy Vector primers T7 and SP6 (Figure 4.2) as well as a microsatellite specific primer (TGTGGCCGC(TG)8,

TATCTTATAT(CT)7, TATCTTATA(GCC)6 or TATCT TATA(GATA)4), such that a double band would be seen on the gel when a microsatellite was present. Reactions were carried out in 20 uL with a profile of 96 °C for 2 min, 29 cycles of 94 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min.

*Mini-prep to isolate plasmid and digest with Eco-RI to verify positive colonies contain inserts*

Plasmid DNA was extracted using a Mini-Prep Kit (Fermentas) and cut using Eco-R1 (Fermentas). Eco-RI was chosen because the pGem-T EASY Vector has a restriction site for EcoRI flanking the insert site producing a product ~25bp long without an insert.

*Sequencing*

Plasmid DNA was then sequenced using the ABI 3730 sequencer and M13 primers (DBS Genomics, Durham UK). Sequences were visualised using BioEdit (Hall 1999) and clones containing suitable repeat motifs and flanking regions were selected for primer design. Out of *c.* 4000 positive clones, 2560 were screened for microsatellite inserts, and 347 of these generated a second band, indicating an insert. Of these, 284 of the appropriate size were sequenced.

*Design primers and amplify in both L. planeri and L. fluviatilis testing for polymorphism*

Primer pairs were designed for 12 loci using Primer3 (Rozen & Skaletsky 2000) implemented in Geneious vR6 (Biomatters). Of these, 7 could be amplified and showed polymorphism. However, several of these were abandoned due to difficulty of allele-scoring, monomorphism or obvious null alleles. The remaining four microsatellite loci used in this study are shown in Table 4.3.

**Table 4.3** Details of microsatellite loci and primers utilised in the study.

| Locus | Primer Sequence (5'–3') | Reference | Allele Size(bp) | Dye |
|---|---|---|---|---|
| LP-003 | F: TCACGTACGCGTTAACTCCA<br>R: TTCCTTAATTGGTCTGCCTCAGGA | (Gaigher *et al.*, 2013) | 88-96 | FAM |
| LP-009 | F: AACTCCCACGTGCAAAATTC<br>R: AGGCATCACTCCTAACGACG | (Gaigher *et al.*, 2013) | 188-194 | FAM |
| Lamper_1 | F:GCGAGTGTCCGAGCAGCG<br>R:TGCGGCACAACCGCGGAC | Developed by Author | 239-269 | FAM |
| Lamper_2 | F:TTACAAGCCACCTTCTCC<br>R: GCTGATGTTGGCAGTGAG | Developed by Author | 154-410 | HEX |
| Lri-5 | F: GCCGACAACAACCAACATC<br>R: CACGCAGGTCACCCTCTAC | (Luzier *et al.* 2010) | 257-278 | TAMRA |
| LP-027 | F: ACAGTCAACCTCCGACATCC<br>R: AGCCCATGATGATTCCATTC | (Gaigher *et al.*, 2013) | 196-208 | FAM |
| LP-028 | F: AGAACTCTGTGGACGTTCCG<br>R: TCTCAAGAAATGAGTTCTCAATCG | (Gaigher *et al.*, 2013) | 231-243 | FAM |
| Lp-046 | F: ACCGCAAACTCATCAGGAAC<br>R: AAGCGGATTTAGAAGCGACA | (Gaigher *et al.*, 2013) | 134-146 | HEX |
| Lamper_3 | F: TGAGGGTCCTGGTGTGCG<br>R: GGCAGGAGGTCACGGGC | Developed by Author | 208-228 | HEX |
| LP-006 | F: TGCCCACACGTGATAGACAT<br>R: GGCGATCGTCATAAATAGCC | (Gaigher *et al.*, 2013) | 114-134 | TAMRA |
| LP-045 | F: AGAGGTGTTTCGCGTGCTAT<br>R: AAGGAGAGAGGAGGTTTCGG | (Gaigher *et al.*, 2013) | 170-185 | TAMRA |
| LP-018 | F: TTAAAAGTGCGGCGAAATCT<br>R: TGTTCCATAACCACTGCTCG | (Gaigher *et al.*, 2013) | 224-238 | TAMRA |
| Lamper_4 | F: TACCCCGTGGCACTTGAC<br>R: TGGACGCCGGAGTTGACC | Developed by Author | 254-542 | TAMRA |

### 4.3.4 Amplification and genotyping of microsatellites

Thirteen recently developed polymorphic microsatellite loci were used to examine the genetic differentiation among and between *L. fluviatilis* and *L. planeri* populations. Eight microsatellite primers developed for European *Lampetra* (Lp-003, Lp-006, Lp-009, Lp-018, Lp-027, Lp-028, Lp-046, and Lp-045; Gaigher *et al.* 2013) one primer set developed for the western brook lamprey *Lampetra richardsoni* (Lri-5; Luzier *et al.* 2010) and four microsatellite primers developed (as described in the previous section, Section 4.3.3) optimised for

European *Lampetra* species (Lamper_1, Lamper_2, Lamper_3, Lamper_4) were utilised in this study (Table 4.3). The locus name, primer sequences, dye, size range, and source references for each microsatellite locus is provided in Table 4.3. Microsatellite loci were multiplex amplified (running multiple loci per lane) using a Qiagen Multiplex kit. Thermal cycler conditions were: initial denaturation at 95 °C for 15 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing temperature 60 °C for 90s and extension at 72 °C for 60s; followed by a final extension at 60 °C for 30 min.

In each primer set, one tenth of the forward primers in each reaction were labelled at the 5' end of the oligonucleotide with a fluorescent ABI prism dye to allow for sizing of the PCR product. ABI prism labels of FAM, HEX and TAMRA were used. The PCR products were then added in specific amounts (0.2 µl for FAM dyed products, 0.3 µl for HEX dyed products and 0.4 µl for TAMRA dyed products) to a 1.625 µl mixture of ABI loading buffer containing ROX labelled DNA size ladder to allow the sizing of PCR products. DBS Genomics (Durham University) ran results in a 3730 ABI DNA Analyser. Visualisation of PCR sizes to a resolution of 1 bp was possible using Geneious VR6 (Biomatters).

Microsatellite loci were tested for null alleles, large allele dropout, and scoring errors due to stutter peaks using MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004). The programme ARLEQUIN 3.5 (Excoffier & Lischer 2010) was then used to test deviation from Hardy-Weinberg equilibrium. Tests for linkage disequilibrium were carried out for each pair of loci using an exact test based on a Markov chain method as implemented in Genepop 4.2 (Raymond & Rousset 1995; Rousset 2008).

### 4.3.5 Genetic diversity and structure

To determine the level of genetic differentiation between pairs of populations, F-statistics (Weir & Cockerham 1984) were calculated for microsatellite DNA loci using ARLEQUIN v 3.5. Significance was tested using 1000 permutations. Allelic richness (calculated for each locus and population) and $F_{IS}$ (inbreeding coefficient) were calculated using the programme FSTAT 2.9.3 (Goudet 1995). STRUCTURE 2.0 was used to assign individuals by genotype to a putative number of populations (*K*; Pritchard *et al.* 2000). $\Delta K$, a measure of the second order rate of change in the likelihood of *K* (Evanno *et al.* 2005), was calculated using STRUCTURE Harvester (Earl & vonHoldt 2012) to assess the highest hierarchical level of structure.

STRUCTURE runs were performed from K=1 to K=12 for the analysis comparing anadromous *L. fluviatilis* and *L. planeri* from paired sites with the inclusion of anadromous *L. fluviatilis* populations form the River Trent. Runs from K=1 to K=3 were performed for the analysis of the Swale populations (*L. fluviatilis* and *L. planeri*) known to be sympatric spawners, as compared to the Ure *L. planeri* population. STRUCTURE runs were performed from K=1 to K=6 for the analysis of *L. planeri* populations, and were performed from K=1 to K=5 for analysis of the Loch Lomond system (with the inclusion of an anadromous population of *L fluviatilis* from the River Nidd, and a freshwater resident population of *L. fluviatilis* from the River Bann for comparison). STRUCTURE was also used with a location prior (LOCPRIOR) to further clarify population structure within the Loch Lomond system (Hubisz *et al.* 2009). For all analyses, four independent runs for each *K* value were performed at 2,000,000 Markov chain Monte Carlo (MCMC) repetitions and 500,000 burn-in and assuming correlated allele frequencies and admixture.

Patterns of microsatellite differentiation were subsequently examined, using a factorial correspondence analysis (FCA) implemented in GENETIX 4.05.2 (Belkhir *et al.* 1996-2004 ), which gives a visual representation of individual genotype clustering. A test for a positive association between genetic ($F_{ST}/(1-F_{ST})$ and geographic distances (isolation by distance (IBD)) based on microsatellite DNA loci was carried out using a Mantel test (10,000 permutations) in Genepop V4.2 (Raymond & Rousset 1995; Rousset 2008). Geographic distances were calculated between sample sites (based on the shortest possible route) using linear referencing tools in Quantum GIS (Lisboa). A Mantel test was also carried out to test for association between genetic distances and number of physical barriers (defined as any feature larger than 0.5 m height at base river level which reaches the full width of the river) between sample sites. Only river systems for which information on barriers was available were utilised in the mantel tests which were: Dee, Wear, and all rivers within the Ouse sub-catchment excluding the Swale due to the low sample size attained for *L. planeri.*

MIGRATE-N (v 3.2.6) was used to estimate levels of historical gene flow between populations (Beerli & Felsenstein 2001; Beerli 2006; Beerli & Palczewski 2010). Pairwise comparisons were carried out between putative species (i.e. *L. fluviatilis* and *L. planeri*) at six locations (Wear, Dee, Lomond, Nidd, Ure, Derwent), of which the latter three are all tributaries in the same river catchment, where samples from both species were available. To implement Bayesian inference in MIGRATE-N, the Brownian motion approximation was selected with an MCMC search of 100,000 burn-in steps followed by 5,000,000 steps with parameters recorded every 100 steps; exponential prior on theta (min: 0, mean: 30, max: 60); and an exponential prior on migration (min: 0, mean: 650, max: 1300). MIGRATE-N was run with parameter values starting from $F_{ST}$-based estimates, and the distribution of parameter values was compared across runs to ensure overlap of 95% C.I.

BAYESASS 1.3 (Wilson & Rannala 2003) was used to estimate the magnitude and directionality of contemporary gene flow between *L. fluviatilis* and *L. planeri*. Pairwise comparisons were carried out for the same six locations that were used in the MIGRATE-N analysis. In contrast to MIGRATE-N, BAYESASS estimates all pairwise migration rates rather than a user-defined migration matrix, and provides unidirectional estimates of *migration* for each population pair. BAYESASS does not assume a migration–drift equilibrium, an assumption that is frequently violated in natural populations (Whitlock & McCauley 1999). In the final analysis, 10,000,000 MCMC iterations were run, of which 1,000,000 were for the burn-in. All other options were left at their default settings. Five to 10 runs with a different starting point were performed for each population pair and results are given as means. The programme TRACER ver. 1.5 (Rambaut & Drummond 2007) was used as a method to qualitatively assess Markov Chain Monte Carlo (MCMC) convergence.

## 4.4 Results

A total of 543 lampreys were genotyped at thirteen loci. Heterozygosity within loci was tested for deviation from HWE, and significant deviations were found for: Lamper_4 in Derwent (*L. planeri*) and Lomond (freshwater-resident *L. fluviatilis*) and Lp_003 in Nidd (*L. planeri*) (Appendix A). These results did not reflect a consistent pattern for a population or locus, and the omission of the relevant loci did not change the overall results. Therefore, all loci were retained for further analyses. Loci were not affected by null alleles for most populations and there were no consistent issues for any given population (Appendix A).

A total of 112 of the 136 $F_{ST}$ values (82.35 %) were statistically significant ($P < 0.05$; Figure 4.3, Table 4.4). $F_{ST}$ values between sites ranged from -0.00524 [Derwent (*L. fluviatilis*) and

Nidd (*L. fluviatilis*)] to 0.191 [Wear (*L. planeri*) and Nidd (*L. planeri*)]. All $F_{ST}$ values between *L. planeri* populations were significant with an average $F_{ST}$ of 0.1285 (± 0.0385), *df* =15 and a range from 0.06045 (Loch Lomond and Derwent) to 0.191 (Wear and Nidd). Only 45.4 % of $F_{ST}$ values for *L. fluviatilis* populations were significant, with an average $F_{ST}$ of 0.0267 (± 0.0345), *df* =55 and a range from -0.00524 (Derwent and Nidd) to 0.11945 (Lomond (freshwater resident) and Bann (freshwater-resident)). When the freshwater-resident *L. fluviatilis* are not included, the $F_{ST}$ values ranged from -0.00524 (Derwent and Nidd) to 0.02537 (Swale and Lomond) with an average $F_{ST}$ value of 0.004184. $F_{ST}$ values between *L. fluviatilis* and *L. planeri* populations ranged from 0.011 (Derwent (*L. fluviatilis*) and Lomond (*L. planeri*)) to 0.18554 (Bann (freshwater-resident *L. fluviatilis*) and Wear (*L. planeri*) with an average $F_{ST}$ of 0.07807 (± 0.04), *df* =66. The two freshwater-resident *L. fluviatilis* populations at Loch Lomond and the River Bann had average $F_{ST}$ values of 0.104356 (± 0.0440), *df* =16 and 0.079511(± 0.0368), *df* =16, respectively, compared against all other putative populations. Average allelic richness per locus ranged from 2.43 (Lp_003) to 14.9 (Lamper_4). Average FIS per site ranged from -0.095 (Wear (*L. planeri*)) to 0.028 (Lomond (anadromous *L. fluviatilis*)).

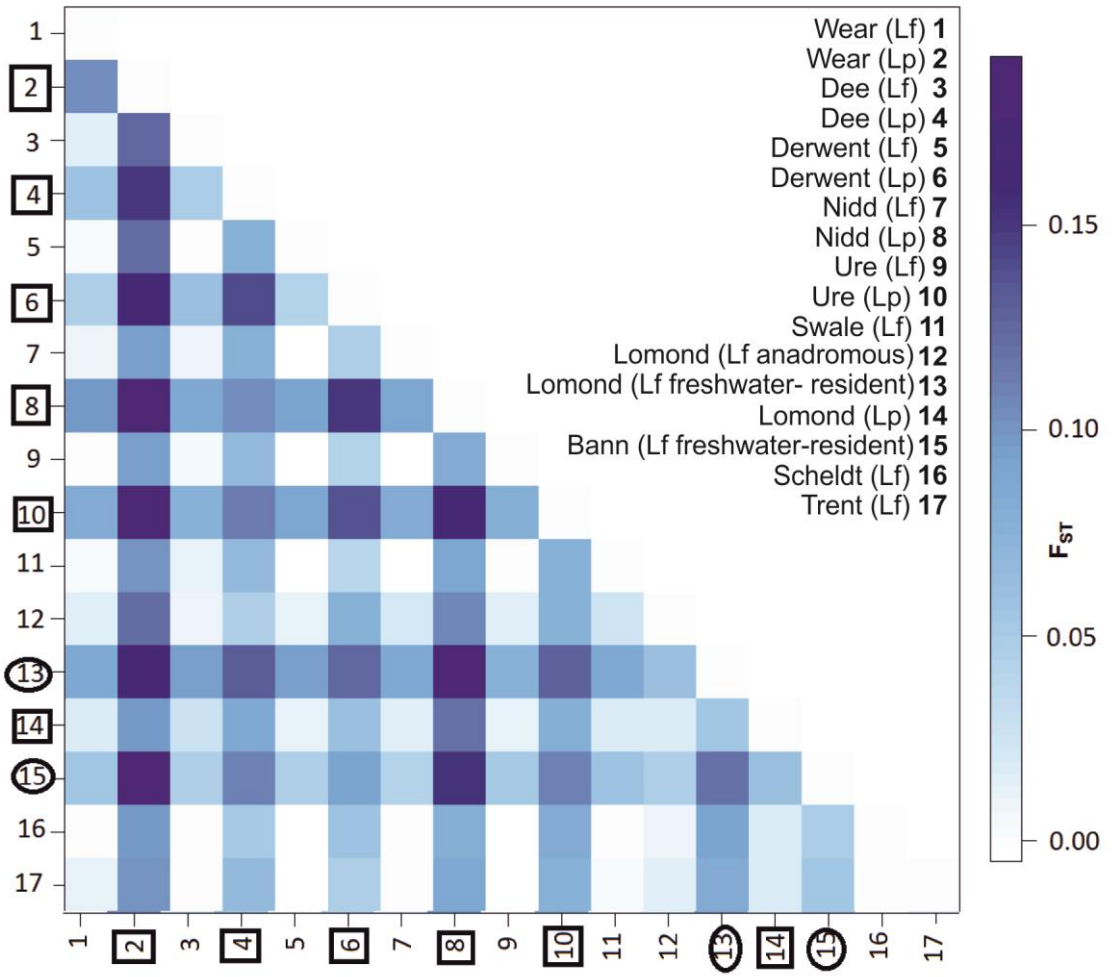



**Figure 4.3** Matrix of pairwise $F_{ST}$ values using 13 microsatellite loci, for all *Lampetra* populations sampled. Where Lf = anadromous *Lampetra fluviatilis*, Lp = *Lampetra planeri,* and Lf Res= freshwater-resident population of *Lampetra fluviatilis*. Numbers on axes are marked with a square to represent *L. planeri* and a circle to represent freshwater-resident *L. fluviatilis*.

**Table 4.4** Pairwise $F_{ST}$ values for 13 microsatellite loci. Numbers in bold indicate a significance level of $P < 0.05$. Boxes outlined with a border signify paired sites. Blue border signifies the Loch Lomond paired site (i.e. three ecotypes). Swale *Lampetra planeri* samples were not included due to low sample size. Where Lp = *Lampetra planeri* and Lf = *Lampetra fluviatilis*. Number 1-17 in the axes represent populations as shown on the right of the table.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | | |
| 2 | **0.10482** | | | | | | | | | | | | | | | |
| 3 | **0.01467** | **0.12577** | | | | | | | | | | | | | | |
| 4 | **0.05688** | **0.14894** | **0.04958** | | | | | | | | | | | | | |
| 5 | 0.00091 | **0.12279** | -0.00055 | **0.0752** | | | | | | | | | | | | |
| 6 | **0.04408** | **0.16403** | **0.06178** | **0.14181** | **0.04307** | | | | | | | | | | | |
| 7 | **0.00721** | **0.09472** | 0.00777 | **0.07476** | -0.00524 | **0.04667** | | | | | | | | | | |
| 8 | **0.09712** | **0.191** | **0.08653** | **0.10329** | **0.09039** | **0.14962** | **0.08821** | | | | | | | | | |
| 9 | 0.00077 | **0.09351** | 0.00525 | **0.0677** | -0.0049 | **0.04353** | -0.00379 | **0.0821** | | | | | | | | |
| 10 | **0.08193** | **0.17794** | **0.07584** | **0.11258** | **0.08824** | **0.13778** | **0.08315** | **0.16067** | **0.07846** | | | | | | | |
| 11 | 0.00296 | **0.10022** | **0.01304** | **0.06757** | -0.00251 | **0.03921** | -0.00298 | **0.08703** | -0.00062 | **0.077** | | | | | | |
| 12 | **0.0153** | **0.12324** | 0.00815 | **0.04645** | 0.01248 | **0.07604** | **0.01961** | **0.10592** | **0.01546** | **0.07703** | **0.02537** | | | | | |
| 13 | **0.08666** | **0.17404** | **0.09354** | **0.13216** | **0.0931** | **0.12483** | **0.08551** | **0.18227** | **0.07546** | **0.12966** | **0.08645** | **0.05919** | | | | |
| 14 | **0.01666** | **0.09681** | **0.02644** | **0.08605** | **0.011** | **0.06045** | **0.01438** | **0.11921** | **0.01153** | **0.07783** | **0.01928** | **0.01688** | **0.05341** | | | |
| 15 | **0.05391** | **0.18554** | **0.04513** | **0.10832** | **0.04628** | **0.09256** | **0.04276** | **0.15284** | **0.05175** | **0.10987** | **0.05899** | **0.04383** | **0.11945** | **0.05999** | | |
| 16 | -0.00082 | **0.0969** | -0.00072 | **0.05025** | -0.00333 | **0.05711** | -0.0003 | **0.07955** | -0.00265 | **0.08349** | 0.00041 | **0.00902** | **0.09156** | **0.019** | **0.04756** | |
| 17 | **0.01178** | **0.09953** | 0.00066 | **0.06703** | -0.0024 | **0.04391** | -0.00148 | **0.08412** | -0.00333 | **0.07572** | 0.00338 | **0.01406** | **0.0824** | **0.01783** | **0.05339** | -0.002 |

Wear (Lf) **1**
Wear (Lp) **2**
Dee (Lf) **3**
Dee (Lp) **4**
Derwent (Lf) **5**
Derwent (Lp) **6**
Nidd (Lf) **7**
Nidd (Lp) **8**
Ure (Lf) **9**
Ure (Lp) **10**
Swale (Lf) **11**
Lomond (Lf anadromous) **12**
Lomond (Lf freshwater-resident) **13**
Lomond (Lp) **14**
Bann (Lf freshwater- resident) **15**
Scheldt (Lf) **16**
Trent (Lf) **17**

STRUCTURE analyses consistently identified *L. planeri* populations as being separate from both *L. fluviatilis* populations (anadromous and freshwater-resident) and each other (Figure 4.4), with an exception being the small sample of *L. planeri* on the Swale, compared to the *L. fluviatilis* population downstream on the same river (Figure 4.5a). Figure 4.4a shows the most likely population structure, among 12 sampling locations in England and Wales, incorporating both species. Six populations were identified ($K$=6 showed the highest $LnP(D)$; Figure 4.6a). *Lampetra fluviatilis* samples appear as a single mixed population. $\Delta K$ = 2 which supports separation of *L. fluviatilis* and *L. planeri* at a higher hierarchical level (Figure 4.6a).

When only *L. planeri* populations were compared, the highest likelihood result identified all populations as distinct (Figure 4.4b). In this case $\Delta K$ was 4 (Figure 4.6b), however, this linked samples from the Nidd with the Dee, and Loch Lomond with the Derwent, which are populations originating from opposite sides of British Isles (see Figure 4.1). When only anadromous *L. fluviatilis* populations were compared the outcome was $K$ = 1. The Loch Lomond system (which contains anadromous *L. fluviatilis*, freshwater-resident *L. fluviatilis,* and *L. planeri* populations) was compared to an anadromous *L. fluviatilis* population (Nidd) and another freshwater-resident *L. fluviatilis* population (Bann). STRUCTURE identified three populations with highest likelihood, while $\Delta K$ was 2 (Figure 4.4c; 4.6c). Using prior location information for the Loch Lomond groups, five populations were identified, however $\Delta K$= 2, showing differentiation at a higher hierarchical level to be between the freshwater-resident *L. fluviatilis* population in Loch Lomond and the other populations (Figure 4.5a & b, 4.6c).

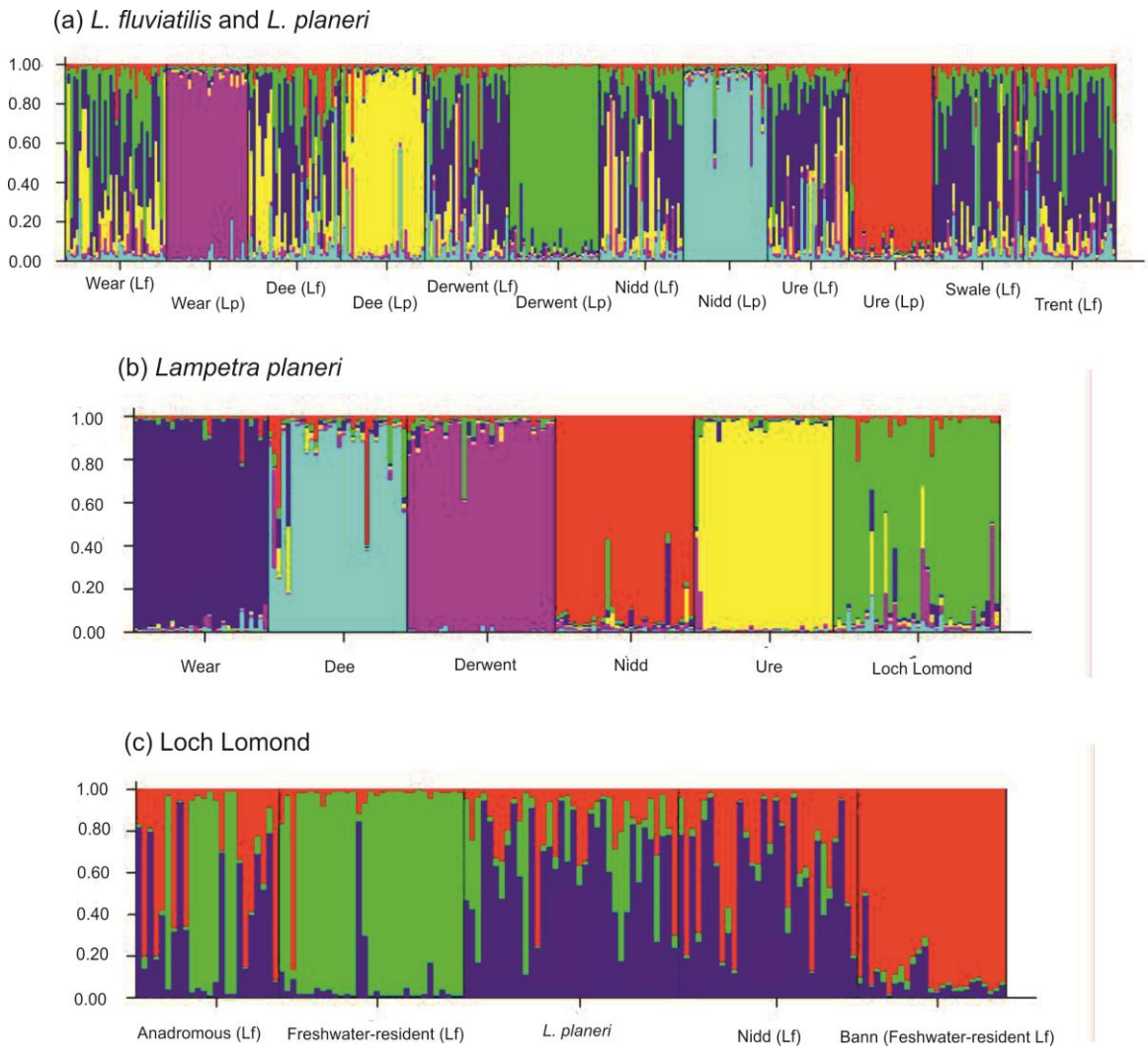



**Figure 4.4** STRUCTURE bar plot generated from microsatellite data for three population clusters of lampreys: (a) Comparison between *Lampetra fluviatilis* and *Lampetra planeri* populations (K=6), (b) *L. planeri* populations (K=6), (c) Loch Lomond populations compared to a population of *L. fluviatilis* from the Humber catchment and freshwater-resident *L. fluviatilis* populations from the R. Bann in N. Ireland (K=3).

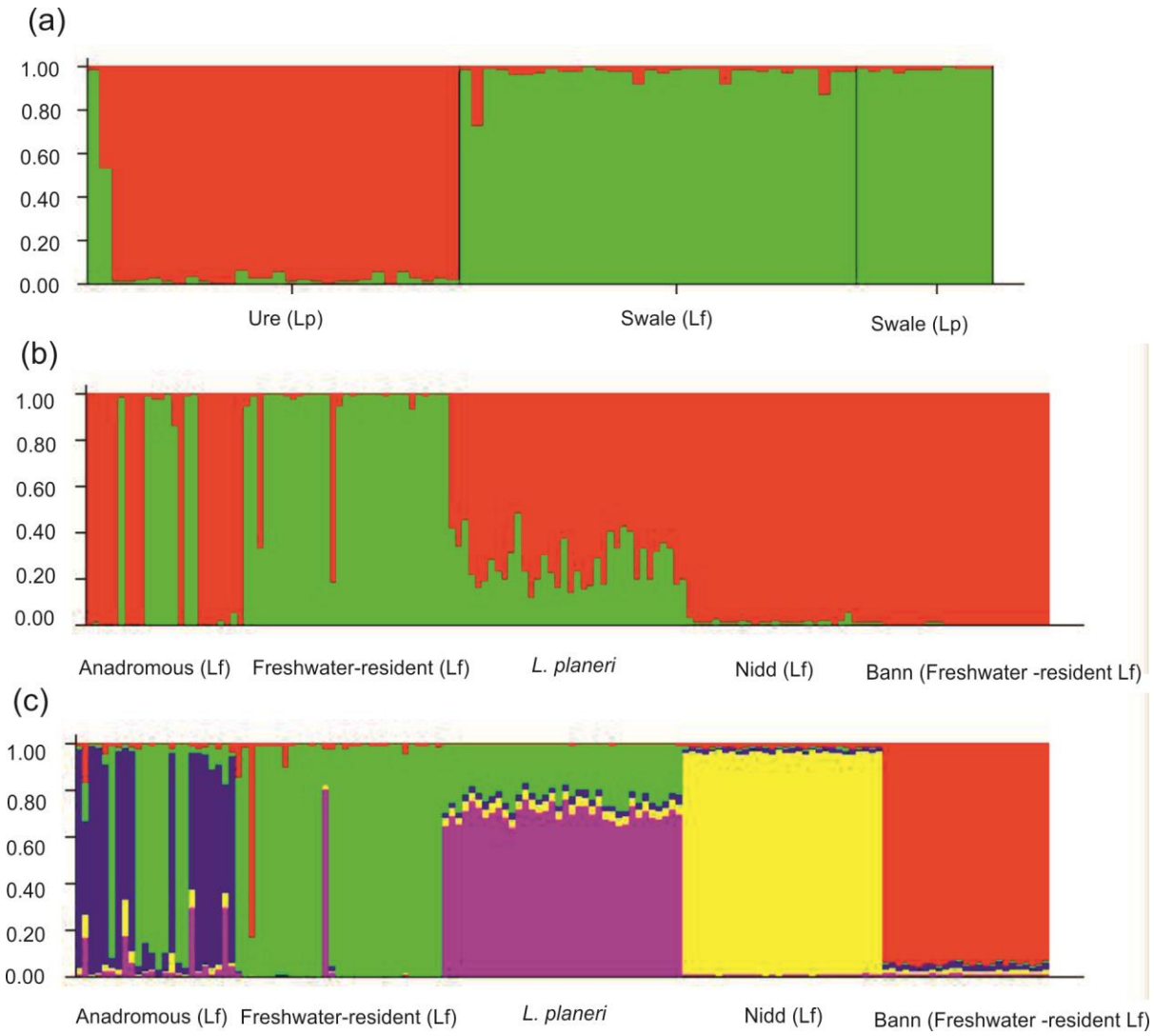



**Figure 4.5** STRUCTURE bar plot generated from microsatellite data showing (a) clustering as inferred from $\Delta K$. $\Delta K$= 2 (*LnP*(*D*)= -2294.8) where Swale Lp is compared to another *Lampetra planeri* population and *Lampetra fluviatilis* from the same river, (b) clustering as inferred from $\Delta K$. $\Delta K$ = 2 in the Loch Lomond system which shows the freshwater-resident Lomond population to be differentiated and (c) $\Delta K$= 5 (*LnP(D)*= -4681) when a location prior is used.

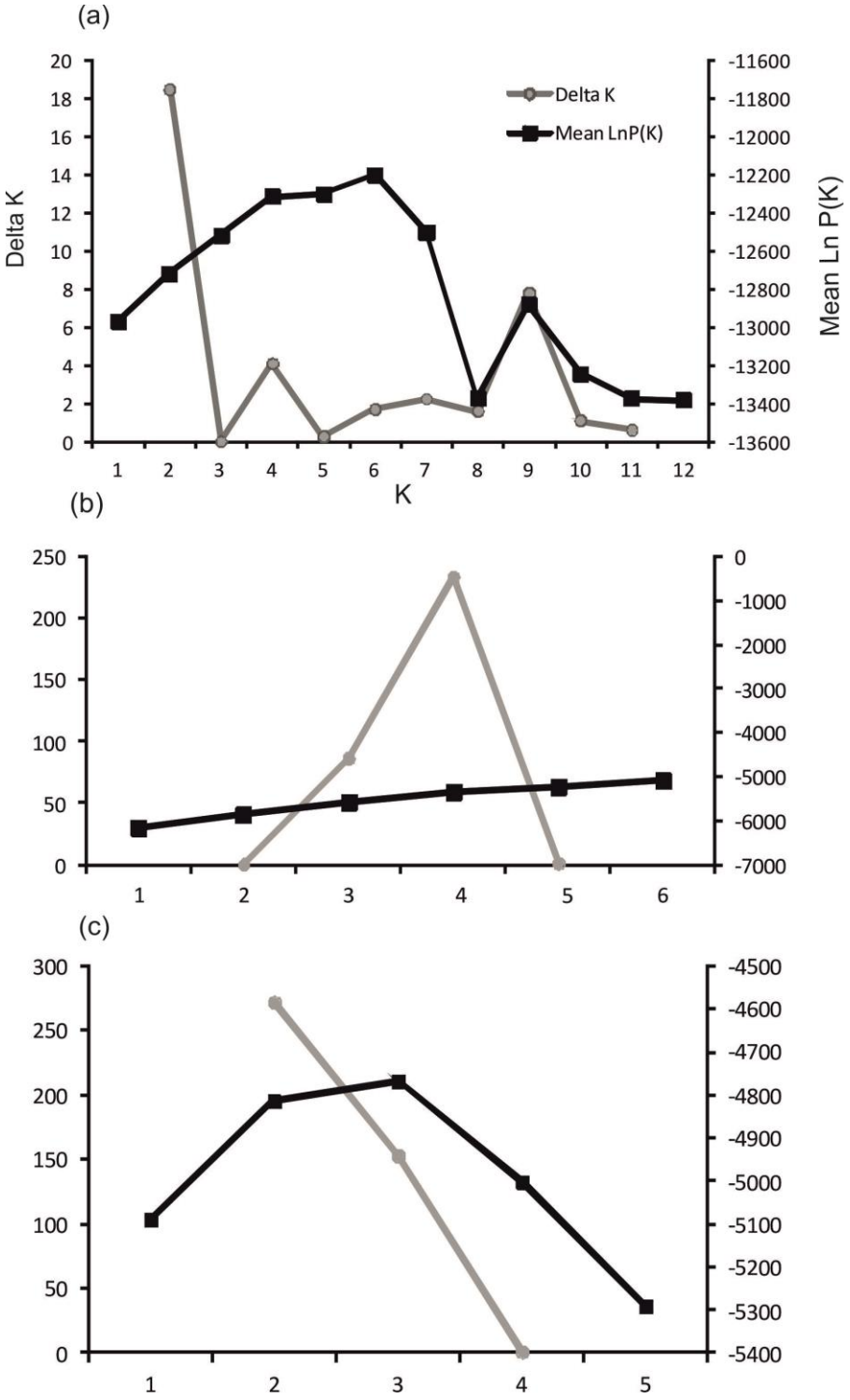



**Figure 4.6** Posterior probability of the data (Ln [P(D|K)]) and values of ΔK (Evanno *et al.* 2005) as a function of K (number of clusters), as resulting from the simulations in Figure 4.4. (a) Comparison between *Lampetra fluviatilis* and *Lampetra planeri* populations (K=6), (b) *L. planeri* populations (K=6), (c) Loch Lomond populations compared to a population of *L. fluviatilis* from the Humber catchment and freshwater-resident *L. fluviatilis* populations from the R. Bann in N. Ireland (K=3).

The FCA plots support essentially the same clusters as identified in STRUCTURE showing *L. fluviatilis* as dominated by one large grouping, with the freshwater-resident populations differentiated (Figure 4.7a) and *L. planeri* populations as all being separated from each other (Figure 4.7b).

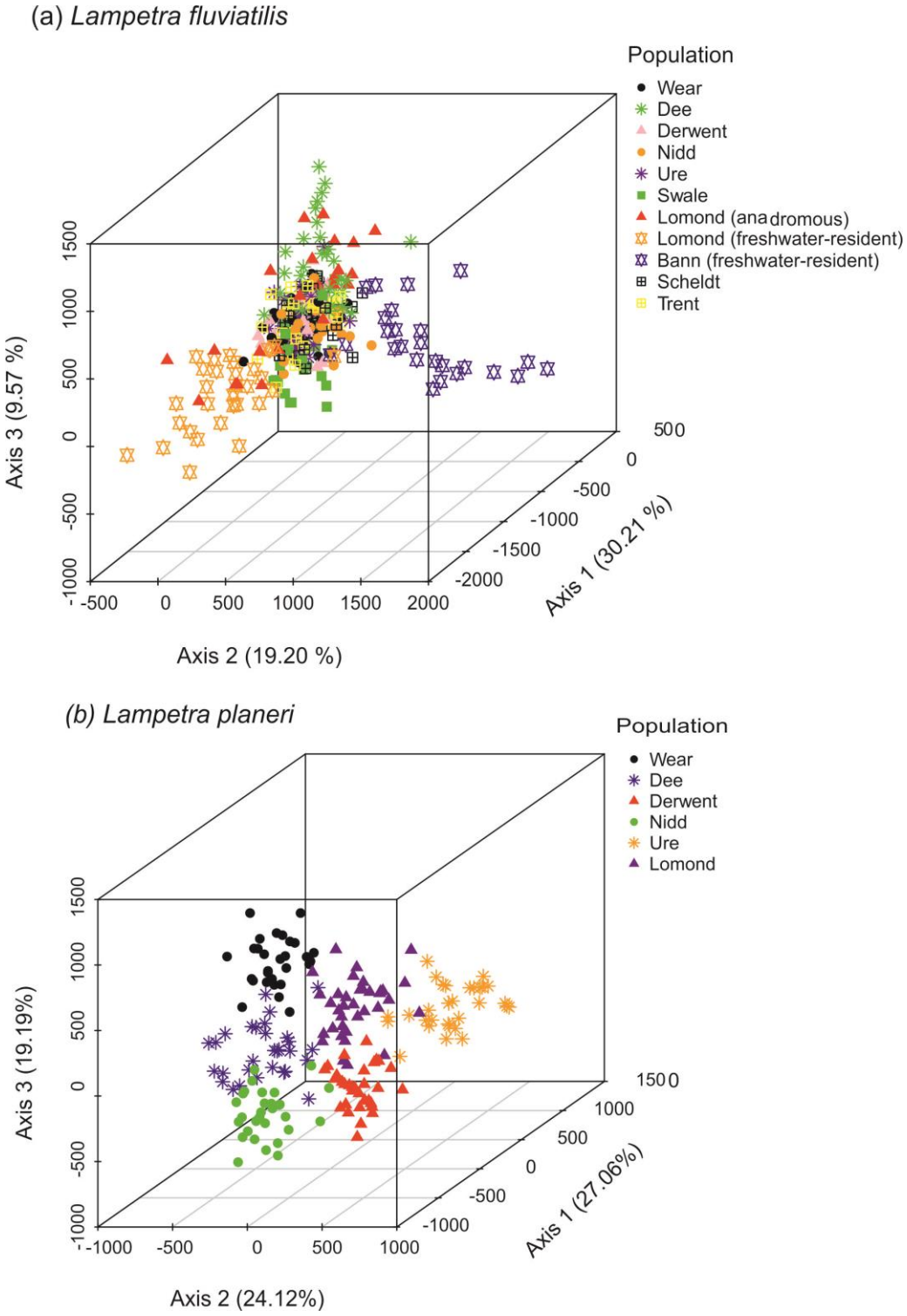
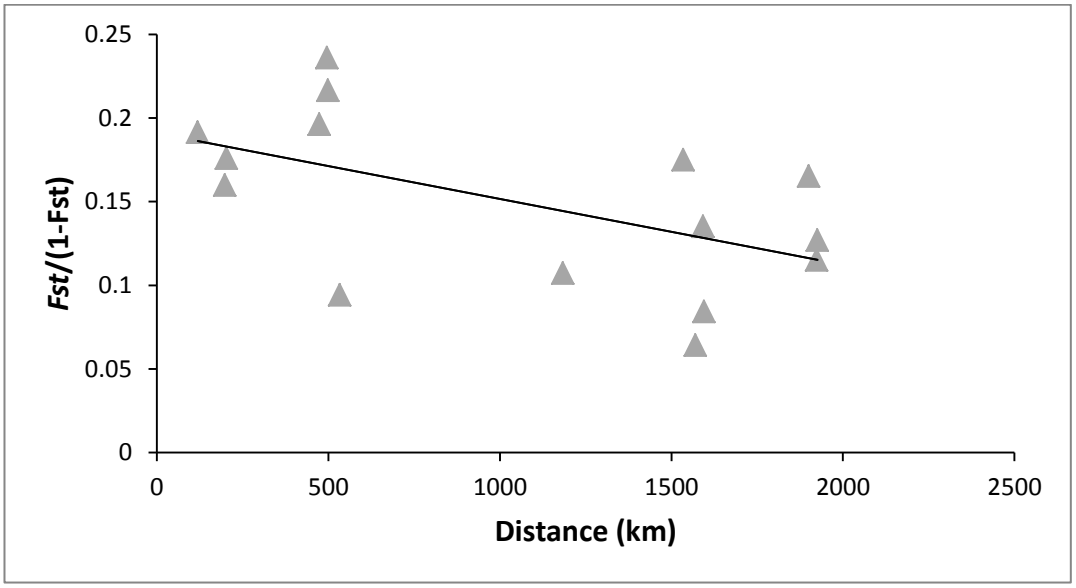


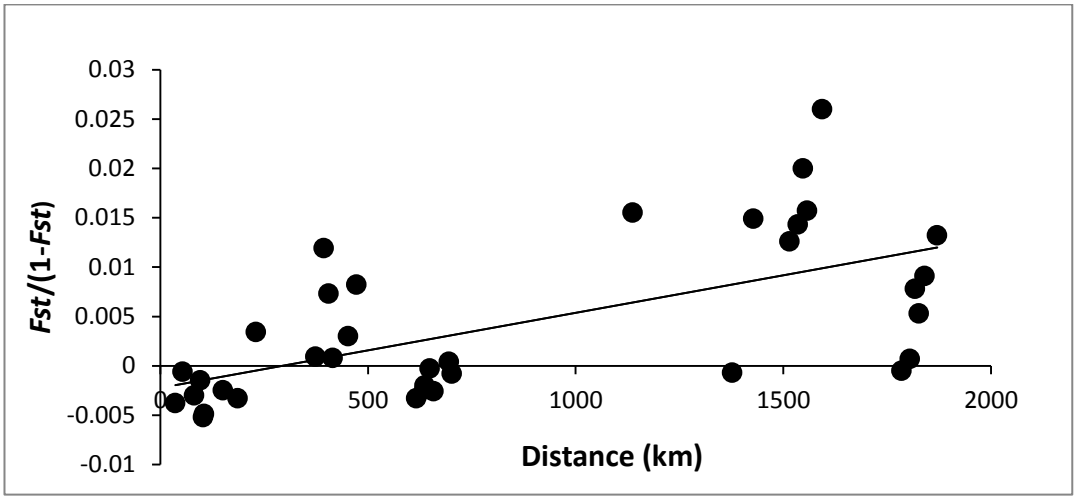

**Figure 4.7** FCA analysis for (a) *Lampetra fluviatilis* and (b) *Lampetra planeri* populations

Mantel tests for correlation between genetic and geographic distance showed a significant negative trend for *L. planeri* populations ($R^2$ = 0.2963; $P$<0.05; Figure 4.8a) and a weak but significant positive linear relationship for all *L. fluviatilis* populations ($R^2$ = 0.0841; $P$<0.05), but when freshwater-resident *L. fluviatilis* populations were excluded (Bann and Lomond) the positive relationship was much stronger ($R^2$ = 0.40, $P$ < 0.0001; Figure 4.8b). Mantel tests examining correlations between genetic distance and the number of barriers along migration/dispersal routes for *L. fluviatilis* and *L. planeri* (populations included are as described in methods), showed a highly significant positive correlation ($R^2$ = 0.8256, $P$ <0.0001; Figure 4.8c).

(a)



(b)




(c)

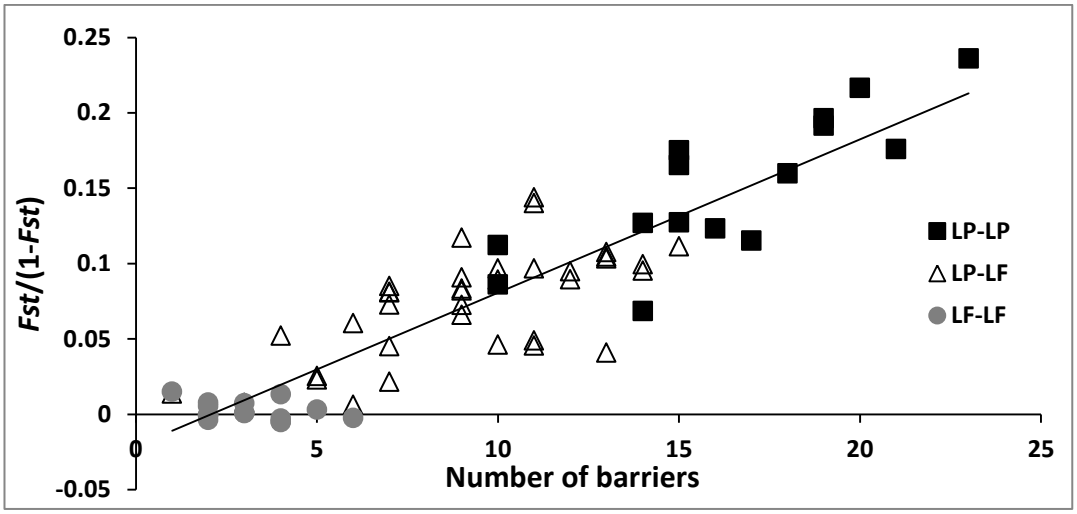



**Figure 4.8** Isolation by distance tests for correlation between genetic differentiation (based on microsatellites) showing (a) geographic distance between freshwater-resident *Lampetra planeri* populations ($R^2$ = 0.30, $P$ < 0.05), (b) geographic distance between anadromous *Lampetra fluviatilis* populations ($R^2$ = 0.40, $P$ < 0.0001) (i.e. excluding freshwater-resident Bann and Lomond Lf. Inclusion of freshwater-resident Lf populations in the analysis reduced the strength of the correlation ($R^2$ =0.0841, $P$ < 0.05) –not shown) and (c) number of barriers between sample sites ($R^2$ = 0.8256, $P$ < 0.0001) where LP-LP signifies comparison of numbers of barriers between *L. planeri* sampling sites, LP-LF is number of barriers between *L. planeri* and *L fluviatilis* sampling sites, and LF-LF is the number of barriers between *L. fluviatilis* sampling sites. Only sites for which barrier information was available were included in the analysis (i.e. Lf and Lp for Wear, Dee, Derwent, Nidd, Ure, and Swale Lf only).

Migration rates obtained from MIGRATE-N, ranged from 3.73 to 10.43 (mean 7.28 +/− 2.36) from *L. fluviatilis* to *L. planeri*, and from 4.18 to 16.28 (mean 10.54 +/− 3.67) from *L. planeri* to *L. fluviatilis*. The six pairwise comparisons (as previously described in section 4.3.1) were assessed and all exhibited significantly asymmetric rates of gene flow based on non-overlapping 95% confidence intervals. Gene flow was always greater in the direction from *L. planeri* to *L. fluviatilis* (which was in the downstream direction for all sites, excluding Loch Lomond). The BAYESASS analysis suggested that there is contemporary gene flow between the putative species and that this is also in the downstream direction from *L. planeri* to *L. fluviatilis* (Table 4.5a & b). Within the Loch Lomond system, gene flow was indicated between all three forms and is at its highest in the direction from both *L. planeri,* and freshwater-resident (Lf), to anadromous *L. fluviatilis*, and from freshwater-resident (Lf) to *L. planeri* (Table 4.5b).

**Table 4.5** Pairwise estimation of current gene flow, M, between (a) populations of anadromous *Lampetra fluviatilis* (Lf) and *Lampetra planeri* (Lp) from within the same river, and (b) populations within Loch Lomond by BAYESASS. Between species gene flow is highlighted in bold. Resident (Lf) signifies the freshwater resident population of *L. fluviatilis* found in Loch Lomond

(a)

| *From* | *To* | M +/- SD | 95% CI |
|---|---|---|---|
| Dee Lf | Dee Lf | 0.007 +/- 0.026 | 0.650 - 0.751 |
| **Dee Lf** | **Dee Lp** | **0.012 +/- 0.011** | **0 - 0.032** |
| **Dee Lp** | **Dee Lf** | **0.300 +/- 0.026** | **0.249 - 0.350** |
| Dee Lp | Dee Lp | 0.988 +/- 0.011 | 0.966 - 1 |
| Wear Lf | Wear Lf | 0.907 +/- 0.051 | 0.807 - 1 |
| **Wear Lf** | **Wear Lp** | **0.011 +/- 0.011** | **0 - 0.032** |
| **Wear Lp** | **Wear Lf** | **0.093 +/- 0.051** | **0 - 0.193** |
| Wear Lp | Wear Lp | 0.989 +/- 0.011 | 0.968 - 1 |
| Derwent Lf | Derwent Lf | 0.871 +/- 0.079 | 0.716 - 1 |
| **Derwent Lf** | **Derwent Lp** | **0.012 +/- 0.012** | **0 - 0.035** |
| **Derwent Lp** | **Derwent Lf** | **0.129 +/- 0.079** | **0 - 0.284** |
| Derwent Lp | Derwent Lp | 0.988 +/- 0.012 | 0.965 - 1 |
| Nidd Lf | Nidd Lf | 0.843 +/- 0.062 | 0.721 - 0.965 |
| **Nidd Lf** | **Nidd Lp** | **0.011 +/- 0.011** | **0 - 0.033** |
| **Nidd Lp** | **Nidd Lf** | **0.157 +/- 0.062** | **0.035 - 0.279** |
| Nidd Lp | Nidd Lp | 0.989 +/- 0.110 | 0.967 - 1 |
| Ure Lf | Ure Lf | 0.956 +/- 0.037 | 0.884 - 1 |
| **Ure Lf** | **Ure Lp** | **0.015 +/- 0.014** | **0 - 0.041** |
| **Ure Lp** | **Ure Lf** | **0.044 +/- 0.037** | **0 - 0.116** |
| Ure Lp | Ure Lp | 0.985 +/- 0.140 | 0. 959 - 1 |
| Lomond Lf | Lomond Lf | 0.761 +/- 0.042 | 0.680 - 0.843 |
| **Lomond Lf** | **Lomond Lp** | **0.017 +/- 0.017** | **0 - 0.051** |
| **Lomond Lp** | **Lomond Lf** | **0.239 +/- 0.042** | **0.157 - 0.320** |
| Lomond Lp | Lomond Lp | 0.983 +/- 0.17 | 0.949 - 1 |

(b)

| *From* | *To* | M +/- SD | 95% CI |
|---|---|---|---|
| *L. fluviatilis* | *L. fluviatilis* | 0.697 +/- 0.024 | 0.650 - 0.773 |
| ***L. fluviatilis*** | **Resident (Lf)** | **0.010 +/- 0.010** | **0 - 0.029** |
| ***L. fluviatilis*** | ***L. planeri*** | **0.011 +/- 0.011** | **0 - 0.032** |
| **Resident (Lf)** | ***L. fluviatilis*** | **0.117 +/- 0.032** | **0.055 - 0.179** |
| Resident (Lf) | Resident (Lf) | 0.969 +/- 0.017 | 0.937 - 1 |
| **Resident (Lf)** | ***L. planeri*** | **0.217 +/- 0.041** | **0.137 - 0.297** |
| ***L. planeri*** | ***L. fluviatilis*** | **0.186 +/- 0.037** | **0.113- 0.259** |
| ***L. planeri*** | **Resident (Lf)** | **0.021 +/- 0.014** | **0 - 0.048** |
| *L. planeri* | *L. planeri* | 0.772 +/- 0.040 | 0.693 - 0.851 |

## 4.5 Discussion

In Chapter 3, it was found that the mtDNA ATPase gene of *L. fluviatilis* and *L. planeri* failed to show any differentiation between the two putative species or among populations, which is consistent with previous studies using mtDNA (Espanhol *et al.* 2007; Mateus *et al.* 2011). However, higher levels of differentiation were found in the Iberian populations of *L. planeri* that may have remained in these refugia throughout the last glacial maximum enabling divergence over time. As glaciers retreated, and northern Europe became once more accessible, anadromous *L. fluviatilis* would have had the opportunity to colonise newly available freshwater habitat and potentially expand into freshwater isolates as potamodromous phenotypes. With expansion into previously unoccupied territory, it is expected that genetic diversity should decrease from the south to the north (Hewitt 1996). This is consistent with the findings here.

In contrast to the mtDNA, microsatellite loci revealed considerable genetic differentiation between European *Lampetra* species. Microsatellite loci exhibited significant $F_{ST}$ values between *L. fluviatilis* and *L. planeri* populations, however, the highest overall $F_{ST}$ values were encountered among populations of *L. planeri.* Conversely, anadromous *L. fluviatilis* populations appeared to be more panmictic, showing little differentiation between the sample locations. Anadromous lampreys (*Lenthenteron* spp.) in Japan (Yamazaki *et al.* 2011), and *P. marinus* in North America (Bryan *et al.* 2005) and Europe (Almada *et al.* 2008), also appear to exhibit similar levels of panmixia, with little or no genetic structure, despite their widespread distribution. Spice *et al.* (2012) found that Pacific lamprey (*Entosphenus tridentatus*) along the west coast of North America showed low but significant differentiation among locations, which is likely due to restrictions in dispersal compared to other anadromous lamprey populations.

The STRUCTURE and FCA analyses corroborate this pattern, showing anadromous *L. fluviatilis* as a single mixed population and *L. planeri* from each sampling location as distinct populations. Overall, this evidence agrees with the original proposed hypothesis, and points to a post-glacial expansion of anadromous *L. fluviatilis* into northern Europe, which prompted the establishment of multiple freshwater-resident *L. planeri* populations that subsequently became genetically differentiated. STRUCTURE analysis clearly shows *L. planeri* to be genetically distinct from both populations of *L. fluviatilis* downstream within the same river, and other *L. planeri* populations. Freshwater -resident *L. fluviatilis* were also found to be genetically distinct from anadromous *L. fluviatilis,* which is also likely to be attributed to these populations remaining within a restricted range throughout their life-cycle compared to the more widespread dispersal of anadromous *L. fluviatilis* that will not necessarily spawn in their natal stream. This also points to multiple independent

divergences of *L. planeri* from an anadromous ancestor (i.e. *L. planeri* are polyphyletic), and geographically proximal *L. planeri* and *L. fluviatilis* populations being genetically closer to each other (i.e. multiple origin hypothesis discussed in Chapter 3, Section 3.1; Figure 3.1b).

The higher level of differentiation between disjunct populations of *L. planeri,* than between *L. planeri* and *L. fluviatilis* is, however, suggestive of some degree of gene flow between *L. fluviatilis* and *L. planeri* since the establishment of these populations. As discussed in the previous chapter (Chapter 3, Section 3.4), gene flow between the putative species may be possible owing to a combination of inter-specific nest association (Huggins & Thompson 1970; Lasne *et al.* 2010) and sneaker male behaviour (Malmqvist 1983; Hume *et al.* 2013c). The proportion of recent immigrants, inferred using a Bayesian modelling approach as implemented in BAYESASS, showed a discernible pattern of directionality in recent migration. The BAYESASS analysis suggests contemporary gene flow and an asymmetric pattern of migration, favouring the direction of *L. planeri* (Lp) to *L. fluviatilis* (Lf). Asymmetric gene flow occurring in these types of freshwater systems can significantly influence the distribution of genetic variation, with downstream populations typically exhibiting higher genetic diversity than headwater populations (Caldera & Bolnick 2008; Morrissey & de Kerckhove 2009; Julian *et al.* 2012). This is supported by estimates of long-term migration among populations from MIGRATE-N, which showed historical migration also to be asymmetrical in the Lp to Lf direction. Yamazaki *et al.* (2011) also found gene flow to exist at multi-temporal scales between potentially sympatric lamprey populations, and suggested ongoing gene flow was the result of imperfect size-assortative mating and plastic determination of life histories.

This observed increase in genetic diversity as one moves downstream towards the lower reaches of the river could result from historical patterns of colonisation, contemporary dispersal reflecting movement bias, fragmented habitat or the presence of dispersal barriers (Morrissey & de Kerckhove 2009; Dehais *et al.* 2010). This asymmetry in gene flow would be expected if Lp populations remain primarily resident further up the catchments with occasional migrants moving further downstream to where they may encounter spawning Lf. Mantel tests for isolation by distance revealed a positive correlation between geographical and genetic distance for anadromous Lf, and a counterintuitive negative correlation among Lp populations. However, while the correlation for Lf was strong (especially when freshwater-resident Lf were omitted), and consistent with expectations (implying that long-range dispersal is less common), the correlation with Lp was weak, and showed a broad range of values for a given distance (see Figure 4.8a). The Lp correlation may, therefore, simply reflect a stochastic pattern or ancestral relationships.

Schreiber and Engelhorn (1998) suggested that anadromous *L. fluviatilis* may mediate gene flow among otherwise disconnected *L. planeri* populations; this would suggest that there would be greater differentiation among *L. planeri* populations that are isolated from *L. fluviatilis*. The number of anthropogenic barriers was found to be significantly correlated to genetic distance between populations and such barriers have been shown to limit the upstream migration of *L. fluviatilis* (Lucas *et al.* 2009). Anthropogenic barriers could therefore be amplifying the isolation of *L. planeri* populations by inhibiting the upstream movement of anadromous *L. fluviatilis* and preventing gene flow mediation in this manner between populations. Meldgaard *et al.* (2003) also detected a statistically significant increase of $F_{ST}$ with the number of weirs between grayling (*Thymallus thymallus*) populations in a Danish river system. Similar decreases of genetic diversity from downstream towards

upstream populations have been observed in other fish species in relation to anthropogenic barriers (Yamamoto *et al.* 2004; Caldera & Bolnick 2008; Raeymaekers *et al.* 2009). Yamazaki *et al.* (2011) found freshwater-resident non-parasitic lamprey populations in the upper regions of dams to be genetically divergent from sympatric anadromous parasitic populations. This pattern is consistent with a scenario where barriers amplify the asymmetry of gene flow from upstream towards downstream sites by allowing passive downstream drift, whilst obstructing active upstream migration. Spice *et al.* (2012) also found that larvae from an anadromous population of *E. tridentatus* at a spawning site upstream of nine dams, which only a small number of adults successfully pass each year, exhibited higher genetic differentiation (i.e. higher $F_{ST}$ values) than most other populations.

The isolation, and subsequent genetic drift, of populations upstream of barriers that have relatively low or no migratory behaviour, is therefore, likely to be magnified by the relative numbers of barriers and their cumulative negative impact on the upstream migration of anadromous migrants originating downstream. The significance of physical barriers for petromyzontid speciation has been previously stressed. (Hardisty & Potter 1971c; Salewski 2003; Yamazaki *et al.* 2011). When the individuals are physically isolated from the source of anadromous parasitic populations, acceleration in genetic divergence results in the subsequent establishment of allopatric speciation (Yamazaki & Goto 2000). It is probable, however, that resident *L. planeri* populations would have become, and tended to remain, isolated without the added anthropogenic hurdles, as there is a natural degree of population separation. This is due to the natural extent of upstream migration in anadromous *L. fluviatilis* which, as previous studies have shown, is usually limited to higher order channels, and individuals do not generally penetrate the smaller brooks even where access is unhindered by barriers (Hardisty & Potter 1971c; Hardisty 1986b). An interruption of gene

flow between different life history forms should consequently stimulate genetic divergence and might ultimately lead to speciation via the development of reproductive isolation between them (Futuyma 1998).

The system in Loch Lomond offers evidence of the potential for gene flow between morphologically differentiated lampreys, as where they are found sympatrically, gene flow between *L. fluviatilis* and *L. planeri* can occur. This scenario is also supported by the lack of evidence for differentiation between the geographically proximal *L. fluviatilis* and *L. planeri* populations on the Swale, although the sample size for the latter population was small (Figure 4.5). This draws similarities to the study by Docker *et al.* (2012), which found no genetic differentiation between silver (*Ichthyomyzon unicuspis*) and northern brook (*Ichthyomyzon fossor*) lampreys occurring sympatrically (also using microsatellite loci), but did however, find differentiation in parapatric populations (i.e. populations that do not significantly overlap but do have adjacent ranges). Yamazaki *et al.* (2011) also found a lack of differentiation between sympatric populations of Arctic lamprey (*Lethenteron camtschaticum*) and its non-parasitic derivatives in the Ohio River, Japan. The STRUCTURE analysis suggests a pattern of connectivity between the Loch Lomond *L. planeri* population and the other regional populations that more closely resembles anadromous *L. fluviatilis* than the other *L. planeri* populations investigated within this study (Figure 4.4). This may be due to the locality from which samples were taken, as both anadromous and freshwater-resident *L. fluviatilis,* and *L. planeri* spawn in close proximity (Hume, *unpub. data*). Consequently, it is possible that anadromous *L. fluviatilis* is mediating regional gene flow, meaning that *L. planeri* at this location exhibit similar diversity to anadromous populations.

The Loch Lomond system also offers unique insight due to the populations of *L. fluviatilis* exhibiting two different migration strategies; one being anadromous, whilst the other is potamodromous, with a more limited migration occurring only between the lake and spawning streams. Alternative life history strategies are common to fishes inhabiting post-glacial lakes, often resulting from adaptation to different foraging strategies or environments (Robinson & Parsons 2002). The STRUCTURE analysis offers evidence that the freshwater-resident *L. fluviatilis* population in Loch Lomond is notably differentiated from the other *Lampetra* populations. As freshwater-resident *L. fluviatilis* do not seem to be derived from the same gene pool as anadromous *L. fluviatilis*, it is unlikely that Loch Lomond represents a partially migrating population. A similar result has been shown in other studies, which have found genetic differentiation between sympatric (or parapatric) anadromous and freshwater-resident lampreys, pointing to a different origin for potamodromrous contingents (Taylor *et al.* 2012).

The BAYESASS analysis suggests that contemporary gene flow is occurring between all three populations in Loch Lomond, indicating a derivation from a similar ancestor. Hardisty & Potter (Hardisty & Potter 1971c) and Hardisty (1986a) have suggested that glaciation played an important role in the evolution of lamprey life history strategies, as at the height of glaciation, migratory routes would have been blocked, favouring the emergence of resident populations. If a refugium existed at Loch Lomond, the resident population would have had more time to diverge (in the same way as the *L. planeri* populations of Iberia) before having contact once again with migratory populations, allowing them to differentiate in allopatry.

The divergence of multiple independent freshwater populations is a common trend in the evolution of diversity for diadromous fish (Schluter & Nagel 1995). However, in this study the geographic scale is small for the extent of differentiation observed. It is apparent, that at an initial stage, there was a post-glacial expansion of anadromous *L. fluviatilis* from southern refugia (Chapter 3) and the subsequent establishment of multiple freshwater-resident *L. planeri* populations. These may have been relatively small founder groups that retained some degree of reproductive isolation that was likely intensified, though perhaps not entirely determined, by the anthropogenic introduction of barriers. Moreover, it was ascertained that there is gene flow between *L. fluviatilis* and *L. planeri* in both long-term and contemporary timescales with an asymmetric pattern of gene flow. This has significant implications for the management of *L. planeri* populations and the extent to which this is underpinned by natural processes will have important evolutionary implications with respect to the mechanisms that generate diversity. These data also strongly support a scenario where multi-temporal and multi-spatial radiation is occurring in the *L. fluviatilis* - *L. planeri* paired-species complex. In contrast to the higher levels of divergence present in the Iberian Peninsula, the northern European populations appear to have been established relatively recently (Chapter 3), and the process of differentiation is still ongoing.

Recognition of new species of lampreys has generally been based exclusively on morphology (Renaud 2011), but increasingly, molecular data is being used to resolve phylogenetic relationships among lampreys (Lang *et al.* 2009; Boguski *et al.* 2012) and to suggest the existence of new cryptic species (Mateus *et al.* 2013a). On a global scale, *L. planeri* and *L. fluviatilis* are considered of 'Least Concern' according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Freyhof & Kottelat 2008a, b, c) and the European Red List of Freshwater Fishes (Freyhof & Brooks

2011). Despite this categorisation, they are generally considered to be endangered within Europe (Renaud 1997; Doadrio 2001; Lusk *et al.* 2004; Bianco & Delmastro 2011). Spatially classifying biodiversity is fundamental for conservation (Margules & Pressy 2000) and, therefore, further investigation utilising the tools of molecular ecology is vital for the survival of these species.

The population structuring of *L. fluviatilis* and *L. planeri* has several implications for their conservation and management. An obstacle in the conservation of these species has been the difficulty of defining appropriate Evolutionarily Significant Units (ESUs), however, this study has revealed that special attention may be required for the management of *L. planeri* populations that are genetically distinct. This would involve assessment on a population by population basis, and considering the results of this chapter, allopatric populations of *L. planeri* may need to be managed as individual ESUs (discussed further in Chapter 5). There is also evidence provided within this chapter to suggest that anthropogenic barriers may be amplifying the isolation (both physically and genetically) of some *L. planeri* populations, making them more vulnerable to extinction events. Therefore, the management and installation of any barriers to migration need to be considered on a cumulative basis within catchments containing lamprey populations (as discussed in Chapter 2).

However, *Lampetra fluviatilis* is not subject to some of the disadvantages that *L planeri* experiences due to their restricted ranges, such as an increased vulnerability to destruction of habitat, competition among kin, and inbreeding (Dieckmann *et al.* 1999). Microsatellites have here revealed that within the Irish Sea and the North Sea, *L. fluviatilis* do not form geographically distinct populations and that, potentially, should be managed as a single

ESU. The lack of philopatry in this species also implies that the translocation of larvae to a particular site has the potential to attract spawning adults (i.e. adults are attracted to larval pheromones) to desired spawning grounds. Whether or not this pheromone is species-specific (within paired- species especially) also has considerable implications, both evolutionarily and with regard to the management of lamprey populations by presenting an application with which to attract spawning adults to new, more suitable, habitat (discussed in Chapter 5).

"Biodiversity must be sustained simply because humans have a moral obligation to ensure the natural, evolutionary existence of species and ecosystems whose values do not depend on their human usefulness." - *Winter* and *Hughes,* 1996.

"If we kill off the wild, then we are killing a part of our souls." — *Jane Goodall*

# Chapter 5: General conclusions and future strategies for conservation

## 5.1 Introduction

Despite the extensive and abundant research carried out to examine the conservation issues surrounding many taxa, fishes still seem to be low down on the priority scale when compared with more traditionally charismatic megafauna. Clark and May (2002) surveyed more than 2,700 taxonomically focused articles that appeared between 1987 and 2001 in the two leading conservation research journals (Conservation Biology and Biological Conservation) and found that coverage was exceedingly uneven across taxa, with a strong bias toward mammalian and avian research. Fishes, which make up nearly half of all vertebrate species, however, were the focus of only 8% of the articles published. This disparity pointed to an important implication; that this bias reflected an even greater bias in funds devoted to research. Unlike conservationists working with, for example, large mammals, who exploit the charismatic nature of the target group, fish conservation tends to be compromised because other resource users often ignore or give little respect to fish which they are unable to visualise, larger fish like salmon and sturgeon being perhaps the notable exceptions (Cowx 2002). Aesthetically appealing species may understandably receive greater attention from the public at large but this, however, does not reflect the holistic approach to the preservation of all biodiversity that some conservationists advocate (Helfman 2007).

Lampreys are about as low as one can get on the charisma scale for a chordate and receive minimal positive press (Helfman 2007). However, in more recent years, the 'peculiar', 'primitive', and even 'ugly' characteristics of lampreys, have worked in their favour and encouraged attention from numerous nature based television programmes, including BBC's 'David Attenborough's Rise of Animals: Triumph of the Vertebrates'. However, the

conservation of lampreys is not often considered within the scope of this type of exposure. Nonetheless, lampreys do offer a unique view of the evolutionary process due to their basal position in the vertebrate lineage and the occurrence of paired species within seven out of the ten extant lamprey genera (Renaud 2011). Most extant species of lampreys are threatened (Renaud 1997) within their range, which is usually (at least in part) due to anthropogenic influences. This continued decline has led to most species being of conservation concern in some context. Efforts encouraged by EU directives (i.e. Habitats Directive) signify that more attention is presently being paid to lampreys than has been in the past. It seems, however, that even within the freshwater sector, there is a general lack of knowledge and awareness of the ecology and conservation needs of lampreys. This differs greatly from the North American perception that sea lamprey *Petromyzon marinus* are pests (Smith & Tibbles 1980). There have been many public campaigns to create awareness of the impacts that this species is having on the ecosystem in the Upper Laurentian Great Lakes and consequently much funding and effort is put into research aimed at their control (Smith & Tibbles 1980; Vélez-Espino *et al.* 2008; Fenichel & Hansen 2010).

Cultural biases such as these can significantly affect management policies (Close *et al.* 2002). However, the loss of lamprey populations from aquatic ecosystems has socioeconomic, cultural and ecological consequences. Lampreys, together with offering a unique insight into speciation and the evolutionary process, remain an important part of the fluvial, lacustrine, estuarine and marine ecosystems and play a vital role in the food web (Close *et al.* 2002) being consumed by predatory fish (Cochran 2009), piscivorous birds (Sjöberg 1989; F. Bracken *pers. obs.* ), and mammals such as otters (McCafferty 2005). For example, on the River Ure, north-east England, spawning lampreys (*Lampetra* spp.) form a strong component of the food provided by grey herons (*Ardea cinerea*) for their newborn offspring,

due to this closely coinciding with the lamprey spawning season (M. Lucas *pers. comm*.). The importance of lampreys in the diet of fish, birds and mammals is almost certainly underestimated due to their cartilaginous skeleton being rapidly digested.

Lampreys also have cultural and socioeconomic value. Native Americans in the Columbian Basin have used Pacific lamprey (*Entosphenus tridentatus*) for food, medicinal, and cosmetic purposes and regard lamprey (*ksuyas*) as a highly valued resource which has resulted in them becoming cultural icons (Close *et al.* 2002). The loss of traditional tribal fishing areas and traditional ecological knowledge surrounding the "eel" (lamprey) due to their decline, threatens the considerable loss of tribal culture (Close *et al.* 2002). For example, here are some tribal legends relating to lampreys:

"I have heard it said that long ago, before the people, the animals were preparing themselves for us. The animals could talk to each other during this time. The eel [lamprey] and the sucker liked to gamble, so they began to gamble. The wager was their bones. The eel began to lose but he knew he could win. The eel kept betting until he lost everything."

- *Tribal legend*

"The Creator told the people that the eels would always return as long as the people took care of them, but if the people failed to take care of them, they would disappear."

- *Ron Suppah,* Vice Chair, Warm Springs Tribes.

Similarly, the pouched lamprey (*Geotria australis*) has been a historical food source, for the Maori, in several of New Zealand's rivers and has been subjected to significant fishing effort in some parts of the country (James 2008). *Lampetra fluviatilis* has also been subject to

a long history of exploitation within European rivers (see Chapter 1, Section 1.3.1) and are marketed either for human consumption or sport fishing bait (Tuunainen *et al.* 1980; Masters *et al.* 2006). Sea lamprey (*Petromyzon marinus*) are considered a delicacy in Portugal and due to the high economic value of this fishery (one animal can cost as much as €50), and consequently, the main Portuguese estuaries and rivers are crowded with fishermen and poachers during the annual sea lamprey spawning migration (Andrade *et al.* 2007). Several other species (*Caspiomyzon wagneri*, *L. fluviatilis* and *Lethenteron camtschaticum*) are also consumed by humans, especially in the Baltic States, Japan, Scandinavia and Russia (Renaud 1997).

Cowx & Portocarrero Aya (2011) noted that current interventions (mostly top-down management activities involving regulations) in inland aquatic ecosystems are not necessarily achieving their conservation objectives, despite a growing body of scientific evidence documenting the need to conserve and protect freshwater biodiversity. Furthermore, the public are not as engaged in freshwater conservation compared with marine, large mammal or bird conservation issues (Cooke *et al.* 2013). Two vital components of aquatic system conservation are: the ability of researchers to communicate information effectively to the general public; and to get the message adequately across to politicians to put these issues on the public agenda, therefore, promoting evidence-based decision-making (Sutherland *et al.* 2004). Within the latter, public support is paramount. Generating awareness of environmental conservation issues among the public is essential if there behaviour is to be altered, to help facilitate informed decisions, and engage governments or regulatory authorities to take action (Cooke *et al.* 2013). Conservation planning has previously succeeded in integrating many empirical disciplines into the pragmatic stakeholder-engaged process of strategy development and implementation.

Nevertheless, challenges remain in the engagement of the social sciences and in understanding the social context of implementation (Reyers *et al.* 2010).

Given the socioeconomic, cultural, and ecological consequences of declining lamprey populations, it is imperative to address declines by implementing effective conservation management. White *et al.* (2005) argue that successful biodiversity conservation is complex, and that changeable political and social landscapes require the inclusion of socioeconomic aspects as well as ecological factors. Biological research, coupled with societal research, can provide not only information about the biological sustainability of any group of animals, but also about the social, cultural, and economic sustainability of industries and communities that rely on them. As human impacts on the environment increase, the link between ecological functioning and societal wellbeing is becoming increasingly apparent (Lubchenco 1998). Therefore, research that charts the change in human attitudes over time (Whatmough *et al.* 2011) provides a measure of how supportive the public may be of conservation management (discussed further in Section 5.8.1).

As outlined in Chapter 1, there are five main threat categories that affect lamprey populations, and most freshwater species worldwide (see Section 1.3, Figure 1.4, and Figure 1.5). This thesis has focused on a subset of threats to lamprey populations which broadly relate to flow modification (as described in Chapter 1, Section 1.3.2, Figure 1.5). The overall aims of this thesis are to offer a holistic approach to the conservation of lampreys, broadly relating to their ecology, evolutionary history and anthropogenic impacts, and to offer some insights as how to better manage them. Outlined below is a discussion relating to the findings from Chapters Two through Five, their implications for conservation, and recommendations for future research focus. Conservation issues encountered by lamprey populations are common to most freshwater species and, therefore, the management

options considered here may be used as a guide for other freshwater fishes. Some conservation issues, however, are more specific to the particular habitat needs of lampreys, and will, therefore, also be applicable to lamprey species other than *Lampetra fluviatilis* and *Lampetra planeri*.

## 5.2 Current protection for lampreys

The tools that are most commonly used in, or proposed for, freshwater fisheries conservation tend to be based on terrestrial management measures (Figure 5.1, adapted from Cowx 2002). These methods usually place emphasis on protected areas, habitat restoration, and stock enhancement programmes that are primarily reactive responses to already well-established issues rather than preliminary measures put in place to avoid these issues occurring at all. The principle of prioritising conservation areas to create nature reserves, or sanctuaries, is well established in terrestrial systems (Cambray & Pister 2002; Crivelli 2002). However, few reserves have been designated specifically for freshwater fishes (Crivelli 2002), but this should change as knowledge of the status and distribution of fish species improves and countries comply to demands to nominate sites of conservation interest under the EU Habitats Directive (Habitats Directive 1992).

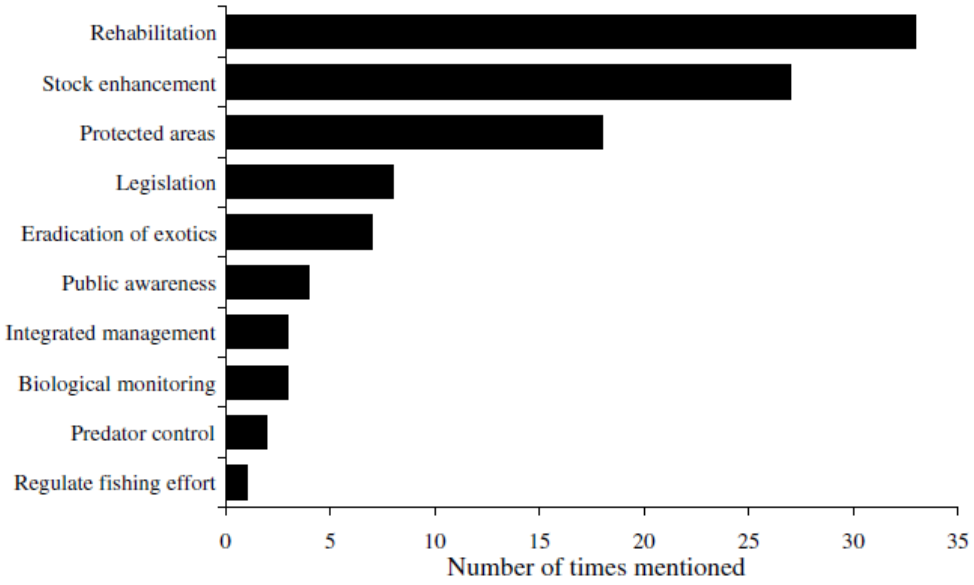



**Figure 5.1** Principal actions used in the conservation of freshwater fish. Adapted from Cowx (2002).

As outlined previously in Chapter 1 (Section 1.1) *Lampetra fluviatilis*, *Lampetra planeri* and *Petromyzon marinus*, are currently listed in the Habitats Directive (Annex II) as species whose conservation requires the designation of Special Areas of Conservation (SACs) (Habitats Directive 1992). Within this thesis, lamprey populations from three designated SACs were discussed: the Endrick Water SAC in Loch Lomond, Scotland, for which the *L. planeri,* as well as *L. fluviatilis* (inclusive of both the anadromous and a freshwater-resident component) are the primary reason for the site selection (Bond 2003); the River Derwent SAC in north-east England for which *L. fluviatilis* is the primary reason for site selection, however (*P. marinus* is also a listed feature), the SAC ends at the confluence with the River Rye where there is a important spawning site for *L. planeri* (however, the upper reaches of the River Rye are under the designation of the Yorkshire Dales national park under which they are afforded protection, as are the *L. planeri*, see Chapter 4, Figure 4.1); and the River Dee in Wales, for which *L. fluviatilis*, *P. marinus*, and *L. planeri* are a qualifying feature, but not the primary reason for the designation of the SAC (JNCC 2007).

Regulatory control is also applied to factors within or outside SACs that are likely to damage the condition of interest features within SACs. In relation to lamprey conservation, for example, these factors would include poor upstream access at barriers (Lucas *et al.* 2009). The criteria for the selection of SACs for lampreys are: sites that hold healthy populations of *Lampetra fluviatilis* (or *Lampetra planeri*), with clear water and suitable areas of gravels, silt or sand for spawning. There are currently eleven SACs in the UK for which *L. fluviatilis* is the primary reason for site selection (Figure 5.2), and nine for *L. planeri* (Figure 5.3). There are also ten SACs for which *L. fluviatilis* is a qualifying feature, but not the primary reason for site selection, and ten SACs exist also with *L. planeri* not being the primary reason for selection (JNCC 2007; Williams & O'Keeffe 2008). There are nine designated SACs in the UK for which *P. marinus* is the primary reason for site selection, and fourteen in which it is a qualifying feature (JNCC 2007). However, there is a lack of connectivity between these SACs as can be seen with the River Derwent (north-east Enland) as a good example. Although the lower reaches of the Derwent protect *L. fluviatilis,* the upper reaches of the river where *L. planeri* populations reside (and the stretches of channel connecting *L. fluviatilis* to *L. planeri*), are not under the protection of the SAC.

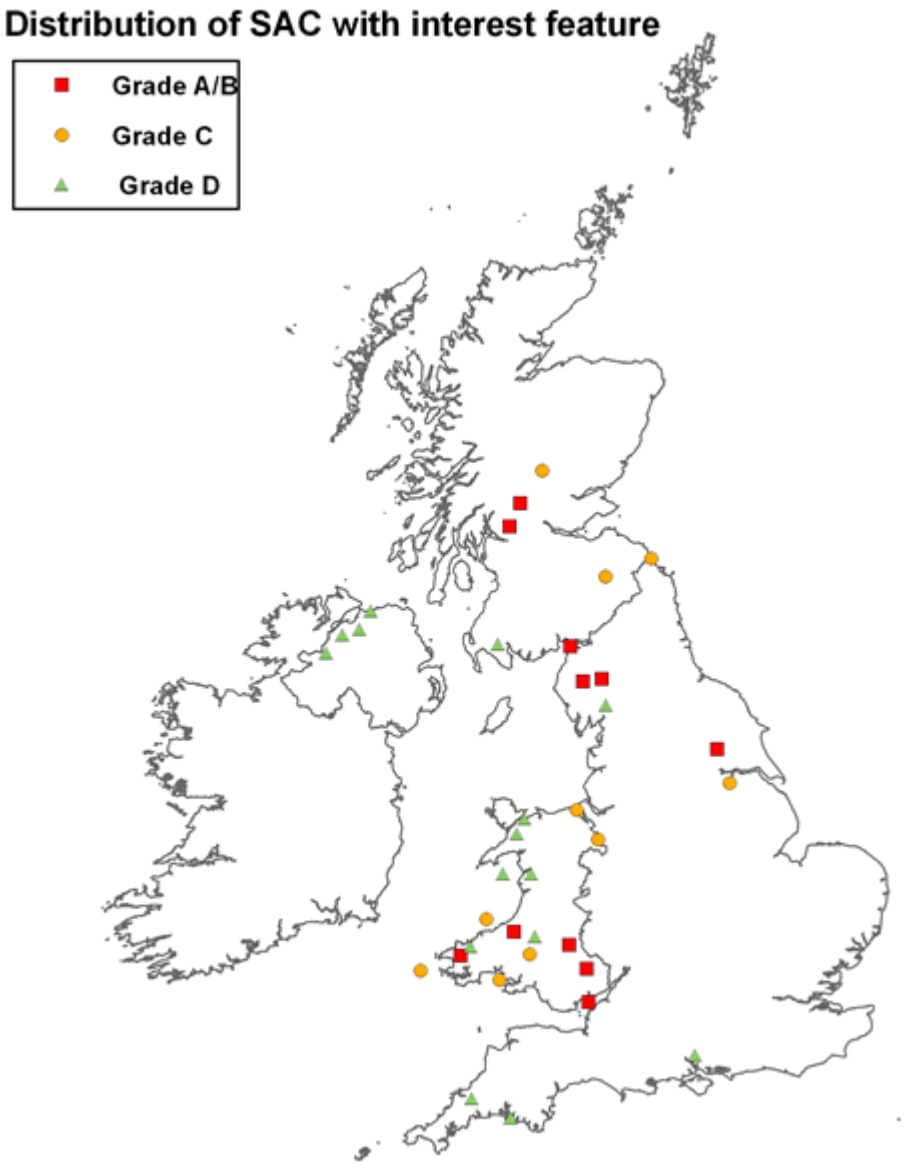



**Figure 5.2** UK Distribution of Special Areas of Conservation (SACs)/Sites of Community Importance (SCIs)/candidate Special Area of Conservation (cSACs) containing *Lampetra fluviatilis*. A= Outstanding examples of the feature in a European context; B= Excellent examples of the feature, significantly above the threshold for SSSI/ASSI notification but of somewhat lower value than grade A sites; C= Examples of the features which are of at least national importance (i.e. usually above the threshold for Sites of Special Scientific Interest (SSSI)/Area of Special Scientific Interest (ASSI) notification on terrestrial sites) but not significantly above this (*Lampetra fluviatilis* are not the primary reason for SACs being selected at these sites); D= Features of below SSSI quality occurring on SACs. These are non-qualifying features ("non-significant presence"), indicated by a letter D, but this is not a formal global grade. Figure adapted from Joint Nature Conservation Committee website (JNCC 2007).

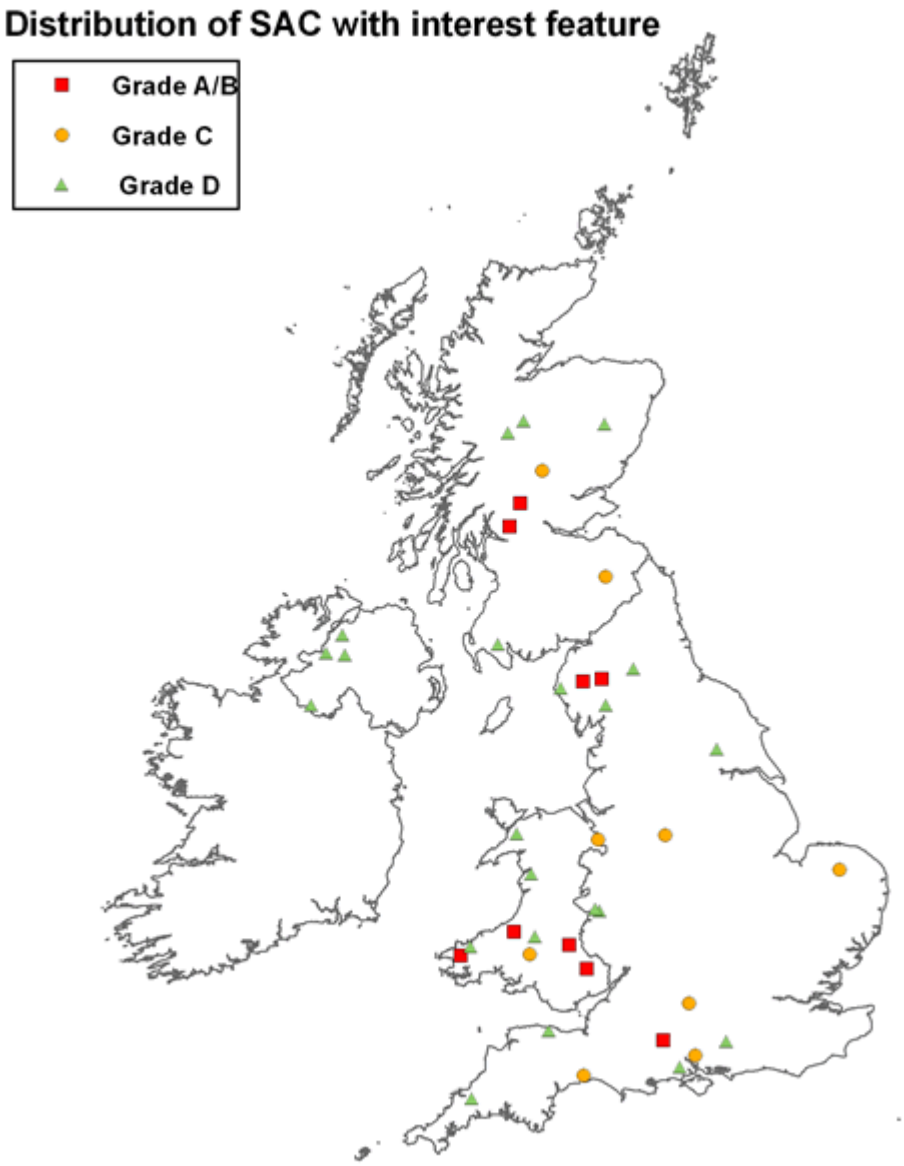



**Figure 5.3** UK Distribution of Special Areas of Conservation (SACs)/Sites of Community Importance (SCIs)/candidate Special Area of Conservation (cSACs) containing *Lampetra planeri*. A= Outstanding examples of the feature in a European context; B= Excellent examples of the feature, significantly above the threshold for SSSI/ASSI notification but of somewhat lower value than grade A sites; C= Examples of the feature which are of at least national importance (i.e. usually above the threshold for Sites of Special Scientific Interest (SSSI)/Area of Special Scientific Interest (ASSI) notification on terrestrial sites) but not significantly above this (*Lampetra planeri* are not the primary reason for SACs being selected at these sites); D= Features of below SSSI quality occurring on SACs. These are non-qualifying features ("non-significant presence"), indicated by a letter D, but this is not a formal global grade. Figure adapted from Joint Nature Conservation Committee website (JNCC 2007).

The Water Framework Directive (WFD), which was implemented in December 2000, is the most comprehensive legislation ever enacted in Europe to address the integrity of freshwater ecosystems and signifies a commitment from countries within the EU to achieve or maintain a 'good ecological status' by 2015. An integral part of achieving this goal is the freedom of movement of fish in both an upstream and a downstream direction. River basin management plans, at both the national and multinational scale, are obligatory under the WFD, and support the process of integrating aquatic resource planning and management, however, the profile of fish conservation needs to be raised and be better integrated into the planning process (Collares-Pereira & Cowx 2004). Whilst the concept of 'good ecological status' is not necessarily synonymous with 'effective conservation', the directive does have much to contribute to the field of aquatic conservation.

## 5.3 Lamprey conservation and management options

Chapter 1 (Section 1.3) identifies five major interacting influences that are implicated in contributing to the decline of freshwater biodiversity, including lampreys, worldwide (Chapter 1, Figure 1.4). These are: pollution, exploitation, flow modification, habitat degradation, and invasive species (Dudgeon *et al.* 2006). Within the life-histories of *L. fluviatilis* and *L. planeri,* there are a number of specific habitat needs vital in completing their life-cycles and allowing them to reproduce (outlined previously in Chapter 1, Section 1.2). Specifically, these habitat needs are: suitable estuarine/marine/lacustrine conditions, free from pollution, with suitable host fish species (*L. fluviatilis* only); a clear migration route from the estuary to the spawning grounds, with suitable river flows and no barriers (mainly *L. fluviatilis* but will apply to *L. planeri* to a lesser extent); suitable spawning areas i.e. suitable hiding places and spawning gravels within freshwater; and slower flowing nursery areas of sand or silt in freshwater for ammocoetes. These ecological traits and life history

characteristics of lampreys, particularly the non-parasitic brook species (i.e. *L. planeri*), make them especially vulnerable. For example, typical gravel spawning habitats are easily disrupted by human activities as gravel is often extracted for road building and other construction practices, and these habitats are often degraded by sedimentation which may be caused by flow modification (Helfman 2007). Larvae spend several years buried in bottom sediments, but in Europe, stream maintenance and flow alleviation programmess, often involve deliberate removal of sediment (e.g. Kirchhofer 1996).

The most pervasive factor contributing to lamprey declines generally falls under the umbrella of flow modification. The necessity for anadromous lampreys to migrate within their life-cycles (and also brook lampreys to a lesser extent) means that they will move through multiple potentially degraded habitats, constituting a gauntlet of environmental obstacles additive in their impact. Barriers to migration can be especially harmful as they can block spawning migrations (few fish passage devices have been designed specifically to accommodate lampreys) and fragment other important habitats required by lampreys. As outlined in Chapter 1 (Section 1.3) these issues (i.e. issues broadly relating to flow modification), which are a subset of the overall factors contributing to lamprey decline, were considered within this thesis. Outlined within this chapter are general conservation threats to lampreys and also concerns which have been highlighted within this thesis. These issues are discussed below and potential management options are outlined along with future recommendations for research focus. These management strategies may be used as guidelines for species with similar habitats needs. The issues addressed within this thesis, and their associated potential management options are summarised in Table 5.1.

**Table 5.1** Summary of conservation issues considered within this thesis surrounding *Lampetra fluviatilis* and *Lampetra planeri*, and the associated potential management options.

| Conservation issue | Management option |
|---|---|
| Lack of information surrounding the distribution and abundance of both *L. fluviatilis* and *L. planeri*, and numbers of spawning adults each year. This contributes to these species being data deficient when assigning a conservation status. | • More baseline surveys specifically targeting lampreys<br>• Surveys of spawning sites including counts of spawning adults<br>• Investigate use of eDNA in assessing presence of lampreys, and population estimates (Thomsen *et al.* 2012)<br>• Investigate use of larval pheromones as a method of monitoring populations (Stewart & Baker 2012)<br>• Designate more SACs based on major spawning sites |
| Increased numbers of hydropower turbines within catchments | • Assess risk at catchment level<br>• Employ the use of lamprey- friendly fish- passes for upstream movement<br>• Use 'fish-friendly' turbines<br>• Use adequate screening to prevent entrainment of transformers and ammocoetes<br>• Locate turbines in 'win-win' sites (Environment Agency 2010) |
| Isolated *Lampetra planeri* populations are genetically differentiated from other *L. planeri* populations and *L. fluviatilis* populations | • Use molecular methods to assess differentiation of each population<br>• Manage anadromous *L. fluviatilis* as a single ESU for the North Sea and the Irish Sea combined<br>• Manage isolated *L. planeri* and freshwater-resident *L. fluviatilis* populations as individual ESUs<br>• Create network of SACs connecting *L. planeri* and *L. fluviatilis* populations |
| Anthropogenic barriers cause fragmentation of spawning habitat and amplify the genetic differentiation of *L. planeri* populations | • Assess numbers of adult lampreys passing barriers<br>• Employ the use of lamprey- friendly fish- passes to aid connectivity<br>• Maintain unobstructed opportunities, within and among populations for genetic exchange, natural dispersal or migration activities, and colonisation of unoccupied portions of habitat<br>• Investigate the potential for creating additional spawning habitat by the addition of gravel beds<br>• Assess potential use of lamprey pheromones to attract spawning adults to appropriate spawning areas (by either translocating larvae or synthesising artificial pheromones). |

## 5.4 Collection of baseline data

If conservation efforts are to be successful, there is an urgent need to carry out research on the basic ecology of species such as population size, and distribution, to strengthen the overall management of species (Cowx & Collares-Pereira 2002). There is little available information about population size and distribution of lampreys in the UK due to two main issues: many surveys that record *Lampetra* spp. are not specifically aimed at lampreys and, therefore, given their specific habitat requirements, are likely to under-record the species; and most surveys concentrate on the juvenile stage, when *L. fluviatilis* cannot be distinguished from *L. planeri*, and therefore, many records cannot be attributed to a specific species. Therefore, there is a need for lampreys to be specifically targeted during biodiversity surveys. An assessment of the numbers of spawning adults (and consequently and estimate of recruitment) is also essential in gathering fundamental data about lamprey populations. Where no adults or transformers identifiable to species are found within populations of interest, molecular methods should be used to establish putative species where possible. Populations with a high likelihood of being *L. planeri,* (i.e. upstream of barriers to migration or in the upper reaches of a catchment) and isolated from anadromous *L. fluviatilis* should be targeted due to an increased vulnerability due to endemism (see Section 5.5.2). However, although the lack of baseline data is an issue, the urgency for direct management intervention for European *Lampetra* spp. is such, that decisions should be based on the best available science and existing experience to initially support management options.

Traditional sampling tools (e.g. netting, traps, electrofishing, visual surveys) can have poor detection limits, because these tools usually have low capture probabilities per target organism, making them reliable indicators of occurrence only for species present at moderate-to-high abundance (Magnuson *et al.* 1994). This implies that non-target species,

which would usually include lampreys, are often overlooked in these types of surveys. However, the monitoring of threatened species through environmental DNA (eDNA) may be a quick, cost-effective and standardised way to obtain basic data on distribution and abundance, enabling efficient deployment of limited conservation resources and taxonomic expertise (Thomsen *et al.* 2012). Thomsen *et al.* (2012) provide evidence demonstrating that a diversity of freshwater species (including amphibians, fish, mammals, insects and crustaceans) can be detected and quantified, based on DNA obtained directly from small water samples of lakes, ponds and streams. Thomsen *et al.* (2012) utilised quantitative real-time PCR (qPCR) and next-generation sequencing, which can provide an index of population size and has a lower detection threshold than traditional sampling tools. Dejean *et al.* (2011) observed that there is rapid degradation of eDNA in surface water, which implyies that the detection of eDNA indicates a very recent presence of an aquatic species.

Environmental DNA detection in wild populations has so far only been applied to a few common or invasive species of amphibians and fish (Ficetola *et al.* 2008; Goldberg *et al.* 2011; Jerde *et al.* 2011). Species specific probes from the mitochondrial genome were utilised within these studies. This potentially poses a problem for lamprey paired-species due to the mitochondrial genome of *L. planeri* and *L. fluviatilis* failing to show any differentiation between the two species (Chapter 3), but could, however, differentiate between *Lampetra* spp. and *Petromyzon marinus*. Nonetheless, eDNA could be utilised to initially detect presence/absence of lampreys and consequently identify sites for further investigation. Alternatively, electrofishing surveys (if the site is likely to have transformers or adult *L. planeri*) or surveys during the spawning season when adults may be encountered, could also differentiate between species. The locality of the detection within a catchment (i.e. lower reaches versus upper reaches of a river) would also offer insight into which species was more likely to be present.

Due to DNA sequencing technology advancing rapidly, costs are also likely to drop (Metzker 2009), making bigger portions of the nuclear genome (e.g. RAD tags) a more feasible option for a species-specific marker for eDNA analysis. Using microsatellite loci (which are part of the nuclear genome) significant genetic differentiation was found between allopatric *L. planeri* and *L. fluviatilis* populations, but not, however, sympatric populations (Chapter 4). If nuclear loci are targeted that demonstrate fixed differences between *L. fluviatilis* and *L. planeri* to 'barcode' and quantify putative species, this method may be applicable. Although microsatellite loci do not show fixed differences between *L. fluviatilis* and *L. planeri,* they may still be applicable to target freshwater-resident populations of *L. fluviatilis* (such as the populations in Loch Lomond and the River Bann discussed in Chapters 3 and 4) from anadromous *L. fluviatilis* populations. The use of eDNA, however, would necessitate rigorous species-specific comparative studies to fine-tune model parameters and further validate the approach in natural freshwater environments before it could be used as an effective management tool.

## 5.5 Flow modification

Freshwater systems facing impending threats, such as significant impoundments at hydropower facilities or dams, are particularly important as research targets. Even if research cannot prevent the construction of new projects, baseline information is a critical first step in assessing effects on lamprey populations (as well as other riverine biota) and thereby influencing future development decisions. In this context, without fundamental information about the overall population size and distribution of lampreys, and the numbers of spawning adults each year (as discussed in the previous section), it becomes almost impossible to make an informed decision about flow modification and its potential impacts.

The introduction of anthropogenic barriers within a river adversely affects the longitudinal connectivity, and also has the potential to alter the surrounding habitat, which may consequently limit spawning or larval habitat available to lampreys (as discussed in Chapter 1, Section 1.3.2). The introduction of hydropower turbines within a catchment also has the potential to affect lamprey populations in the same way that barriers and dams do, but creates additional problems for downstream moving lampreys due to the potential for impingement on screens and entrainment within the turbines themselves (Chapter 1, Section 1.3.3). Discussed in this section are the effects that flow modification, in the form of barriers or hydropower turbines, may have on lamprey populations and potential options for the mitigation and management of these issues. These issues are common among many aquatic species, and therefore these guidelines may be used a framework for the conservation and management of other fishes.

### 5.5.1 Hydropower turbines

Archimedes screw hydropower turbines (which are deemed to be 'fish friendly') were found to have a low effect on downstream moving lamprey passage (Chapter 2). However, the cumulative impacts of these turbines within a catchment must be considered. Even where the effects at one site or design are minor, future developments need to take into account cumulative within-catchment impacts as well as site-specific impacts. Due to policies encouraging the 'freedom of movement' within rivers such as the WFD, mitigation measures at barriers are usually considered for upstream migrating biota (which are not always effective for lampreys), in the form of fish passes (Nunn & Cowx 2012; Foulds & Lucas 2013). There are, however, no such measures in place to support the passage of downstream moving lampreys at hydropower schemes. In the incidence of downstream moving lampreys being severely affected, future populations could ultimately be severely

bottlenecked. Therefore, any benefits gained by facilitating adult lamprey upstream passage at barriers could be negated by losses of larval and juvenile lamprey.

The notable increase in the number of hydropower schemes in recent times is in response to policies of encouraging renewable energy and reducing reliance on fossil fuels (See Chapter 2, Section 2.1), and therefore, foreclosing all hydropower developments is not an option for policymakers. Consequently, the best way to mitigate this threat to lampreys is to make recommendations for locating projects where they will do the least harm (Abell 2002). The Environment Agency (2010) in the UK advises that hydropower development should be concentrated in severely degraded areas, in the context of the WFD, and that 'win-win' sites should be identified. 'Win-win' are defined as sites in which the introduction of a hydropower scheme, and at the same time the introduction of a fish pass at sites which already have barriers such as weirs, could deliver an improvement in the local environment (by increasing the connectivity with a fish pass) as well as renewable electricity. Hydropower development should, therefore, be focused on channels that are already severely degraded/impounded by existing weirs/barriers, and which might actually have their accessibility improved by the addition of fishways. This seems wise, particularly while efforts are made to generate the knowledge needed to minimise potential environmental damage from low-head hydropower facilities in ecologically sensitive catchments and sites.

Small-scale hydropower developments in higher order river channels, generally have greater potential to impact diadromous fishes (i.e. more fish are likely to pass through these sections of river, than they would at sites in the upper reaches of a catchment), and therefore, development in these locations should also be limited. Similarly, the installation of hydropower developments is also likely to have less impact on lamprey populations if

they are placed in lower order rivers (preferably not within a designated SAC for lampreys) due to the relatively lower number of lampreys that have the potential to pass through turbines compared to the lower reaches of the river. As discussed in Chapter 2 (Section 2.5.1), the relative diel and seasonal patterns of migration in lampreys will ascertain the potential impact of a hydropower turbine at differing times. If for instance, hydropower turbines are placed in SACs designated due to the presence of *L. fluviatilis* (e.g. River Derwent, north-east England), the running of turbines during peak emigration periods, especially at night time (due to lampreys being negatively phototactic), has the potential to have a greater impact on the lamprey population than it would if the turbine was run during daylight hours (Bracken & Lucas 2013). Therefore, if it is necessary to introduce hydropower turbines to a river system with an important lamprey population (especially within SACs for lampreys) the timing of the peak period of emigration and drift should be taken into account when considering how best to reduce the impacts on lamprey. This mitigation should also be employed within SACs for other species and guidelines should be based around any peak periods of movement/migration.

The ability of larval pheromones to attract migrating adults (See Chapter 1, Section 1.7) may become relevant to managing barriers and hydropower turbines. If attraction to the relative components of larval odours is common within all lamprey species, the applications of these pheromones (by either the introduction of larvae or the production of synthetic pheromones) could prove to be a practical management application to redirect migrating adult lampreys away from hydropower schemes and towards more accessible spawning habitat (this is discussed further in section 5.6.1). The installation of hydropower facilities within a river also has the potential to fragment habitat and consequently isolate populations. Where gene-flow is limited, or migration is limited, isolated populations may

consequently become genetically differentiated (as seen with *L. planeri* in Chapter 4), which has considerable implications for their conservation due to the increased endemism of these populations. These issues are further discussed in the next section (5.6.2).

## 5.6.2 Genetic differentiation, and the role of anthropogenic barriers, within lamprey populations

### *Genetic differentiation*

Using microsatellite loci, significant genetic differentiation between *L. planeri* and *L. fluviatilis* populations was found, which is in direct contrast to the lack of structuring found using mtDNA (Chapters 3 and 4). Mitochondrial DNA markers showed *L. fluviatilis* and *L. planeri* to exhibit little phylogeographical composition compared with the Iberian Peninsula, much further south, where populations exhibit significant structuring (Chapter 3). Analysis using microsatellite markers however, revealed the most notable differentiation to be among *L. planeri* populations, whilst anadromous *L. fluviatilis* populations exhibited very little differentiation between sampling sites. Docker (2009) suggested that isolated populations of brook lampreys that are genetically very distinct may represent cryptic species. Within the Iberian Peninsula, *L. planeri* has been shown to represent a complex of cryptic species (*Lampetra alavariensis* sp. nov., *Lampetra auremensis* sp. nov., *Lampetra lusitanica* sp. nov.; Mateus *et al.* 2013a) that do not overlap geographically and may have diverged in an allopatric manner similar to the pattern exhibited within the British Isles. There is a substantial chance, therefore, that the number of brook lamprey species in the genus *Lampetra* may be underestimated, due to differentiated populations often being considered the same species due to their relatively conserved body form (Martin & White 2008; Boguski *et al.* 2012).

Due to the relatively recent expansion of *L. planeri* within Northern Europe (as discussed in Chapter 3, Section 3.5), isolated *L. planeri* populations have not yet diverged to the extent to which they have in the more ancient populations in the Iberian Peninsula (see Chapter 3, Figure 3.6). It is, therefore, possible that speciation has not yet reached completion as reproductive isolation mechanisms are not yet present between *L. fluviatilis* and *L. planeri*. It seems that paired species of lamprey tend to exist along a continuum that is dependent on the isolation of the population in question. In this study, *L. planeri*, originating from sites with sympatric populations of *L. fluviatilis,* did not exhibit the same pattern of genetic differentiation that was exhibited by *L. planeri* in more isolated locations. This implies that where *L. fluviatilis* is present, continued gene flow between both parasitic and non-parasitic forms will occur (Chapter 4, Section 4.5). Whether the ecological differences between lamprey species- pairs are associated with distinct gene pools, or whether environmental factors trigger a divergent adult phase, there is not likely a single answer for all lamprey species- pairs (Docker 2009).

As discussed previously, surveys collecting baseline information on lamprey populations are a vital starting point for conservation efforts (Section 5.4). However, this information is only valuable in combination with an accurate portrayal of the taxonomic status of paired lamprey species, due to conservation efforts relying on documenting species-specific declines in abundance and distribution. For example, if all *L. planeri* populations are labelled as *L. fluviatilis*, due to larvae being indistinguishable, management strategies will not accurately incorporate the specific life-history needs of *L. planeri*. Genetic information is instrumental in the implementation of species identification, assessment of population structure, and estimation of genetic diversity (Ward 2000). The assessment of biodiversity within and among populations is central to identifying and prioritising areas for

monitoring, management and protection, and the main goal of management should be to maintain levels of gene flow and maximum genetic diversity, as inferred from molecular data (Moritz & Faith 1998; Crandall *et al.* 2000).

The inability to distinguish between the larval stage of paired species has considerable conservation implications as it becomes a barrier to obtaining an understanding of current species distributions (Docker 2009). The development of an affordable genetic tool to enable identification of larval samples to species would, therefore, be an invaluable tool for managers. This thesis has shown that microsatellite markers are capable of doing this to the extent of identifying whether larvae come from a population, that exhibits considerable gene flow with anadromous *L. fluviatilis*, or whether it is isolated, however, more powerful markers may be needed to resolve this any further (e.g. RAD tags as used by Mateus *et al.* (2013b). It is arguable, however, whether or not it actually matters whether larvae are to become *L. planeri* or *L. fluviatilis*. The importance of a population from a management perspective, should be more focused on whether a population is part of the more panmictic gene pool exhibited by anadromous *L. fluviatilis* (and their sympatric populations of *L. planeri*), or whether it has become isolated, and consequently differentiated as separate Evolutionarily Significant Units (ESUs). Anadromous *L. fluviatilis* around the North Sea and Irish Sea, were shown to be a single mixed population and should therefore be managed as one. This seemingly cosmopolitan single population of *L. fluviatilis* is also potentially mediating gene flow (Chapter 4, Section 4.5) with these isolated freshwater-resident populations and, therefore, it is essential to maintain this dynamic by providing freedom of movement for anadromous lampreys.

Care must be taken, however, as the designation of too many ESUs (over-splitting) could lead to unnecessary waste of management resources and can occur for example, if two ESUs are assigned due to statistically significant differences in allele frequencies but this differentiation is not associated with biological differences. This potentially becomes more of a problem as more highly polymorphic markers are used (Allendorf & Luikart 2007). Therefore, many kinds of information should be integrated when identifying a ESU such as, life-history traits, environmental characteristics, phenotype divergence, and patterns of gene flow (Figure 5.4, adpated from Moritz *et al.* 1995).

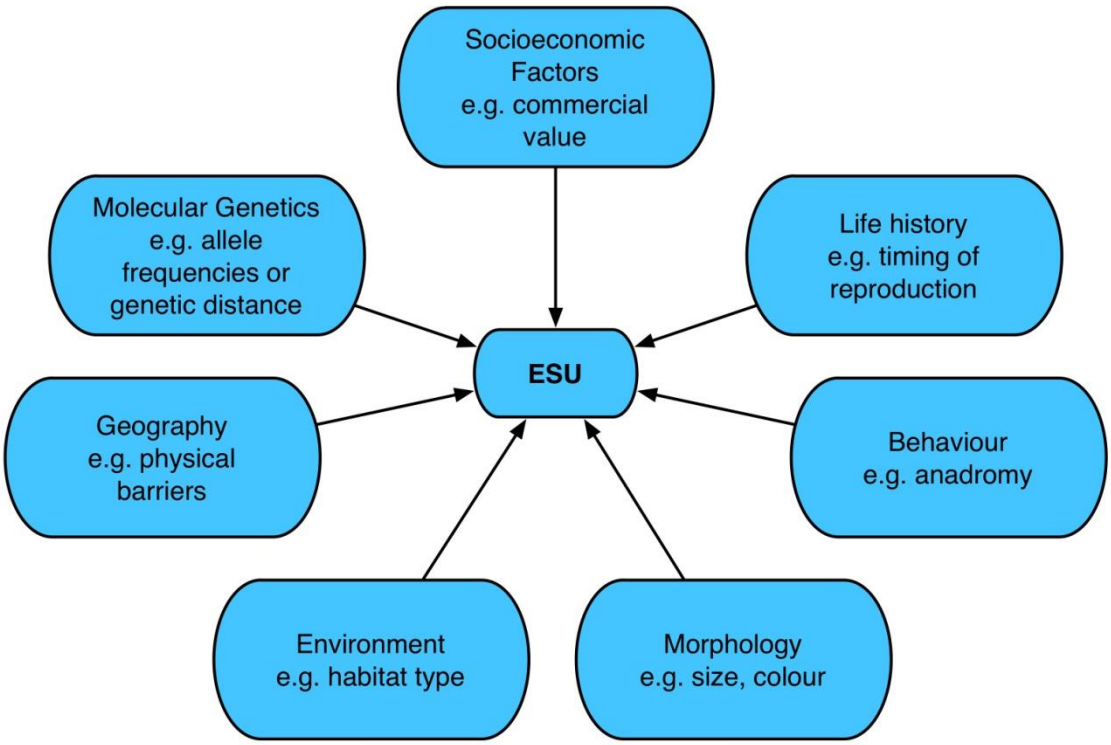



**Figure 5.4** Sources of information that should be utilised in designating a population (or group of populations) as an Evolutionarily Significant Unit (ESU). Modified from Moritz *et al.* (1995).

Genetically differentiated populations such as *L. planeri*, and freshwater-resident *L. fluviatilis* populations (Chapter 4, Section 4.5) should be managed as separate ESUs as has been

suggested with the identification of four distinct phylogenetic clades of *L. planeri* in the Iberian peninsula (Mateus *et al.* 2013a). All habitats utilised by these populations should be consequently evaluated and adequate protection assigned (perhaps listing these populations as the primary feature of a newly designated SAC or SSSI). *Lampetra planeri* however, have attracted relatively little conservation attention in previous years, presumably as a result of their seeming widespread presence within the UK, and were not included in the UK. Biodiversity Action Plan, despite the inclusion of *L. fluviatilis*. Certainly there is provision for protecting infraspecific diversity in current legislation as the guidelines for designating Sites of Special Scientific Interest, for example, state that genetically distinctive fish populations are considered during the selection of suitable sites.

*Anthropogenic barriers*

Anthropogenic barriers appeared to amplify the pattern of genetic differentiation exhibited in *L. planeri* populations (Chapter 4, Figure 4.8). Gene flow was consequently found to be asymmetric due to the barriers allowing downstream movement, enabling gene flow from *L. planeri* to *L. fluviatilis,* whilst obstructing active upstream migration, and gene flow from anadromous *L. fluviatilis* populations to *L. planeri* populations further upstream (Chapter 4, Section 4.5). This physical isolation of *L. planeri* populations may accelerate their allopatric divergence and consequently cryptic speciation. The conservation of cryptic complexes requires special consideration due to the fact that species, that are already considered to be threatened, might actually be composed of multiple species (Bickford *et al.* 2007; Scheffers *et al.* 2012). The catalogue of cryptic species has grown exponentially over the past two decades (Bickford *et al.* 2007; Pfenninger & Schwenk 2007), with 60% of newly described species now derived from cryptic complexes (Ceballos & Ehrlich 2009) creating a whole new dimension to conservation issues. This reinforces the case to manage genetically distinct brook lamprey populations separately (as distinct ESUs) for the conservation of

their biodiversity. This would maximize their need for protection considering the very limited distribution of these populations.

The isolation of these populations of *L. planeri* by anthropogenic barriers violates the 'freedom of movement' within water bodies as specified within the WFD. Management should, therefore, focus on unblocking the migration routes leading to these isolated populations, so that adult and juvenile migration can be resumed. Unblocking can be accomplished by either the removal of barriers and weirs (or hydropower turbines, Chapter 2), or the construction of functional fish passages in rivers where spawning and larval habitats are situated. If the increased genetic differentiation of *L. planeri* populations, even within tributaries of the same catchment or sub-catchment (i.e. the Ouse, north-east England), is at least in part the result of anthropogenic barriers, the removal of barriers will effectively dilute their biodiversity. It may, therefore, be argued that this is not in fact the best way to manage these populations. It will, however, increase the chances of conserving these genes within the largely panmictic lamprey population, and reduce the likelihood of these populations being wiped out by extinction events due to their endemism.

In some respects, the ongoing gene flow between *L. planeri* and *L. fluviatilis* (outlined in Chapter 4, Table 4.5) provides a built in contingency for the conservation of *L. planeri* populations. In the occurrence of habitat loss, or the downstream displacement of individuals due to a flooding event for example, *L. planeri* still exhibit the capability of reassimilating with *L. fluviatilis* populations, rendering individuals capable of passing on gametes instead of the biodiversity within these populations being completely lost. To conserve lampreys existing along the freshwater-resident- anadromous spectrum, particular effort needs to be made to assess 'mixing zones' between species as well as isolated populations. In this context, the designation of SACs for isolated lamprey populations

should extend and connect the existing network of nature reserves, rather than create a large number of fragmented protected areas. For example, within the Humber catchment (see Chapter 4, Figure 4.1), there are two designated SACs which are on the River Derwent and the Humber estuary. However, the resident *L. planeri* populations, which were found to be significantly genetically differentiated in Chapter 4 of this thesis, do not occur in these SACs, and are therefore, not afforded any protection. In this sense, the statutory SACs in fact, seem to be of little consequence to these *L. planeri* populations as they do not match their distribution or facilitate connectivity with *L. fluviatilis* populations.

The key conservation issue is ensuring the connectivity of habitats. Therefore, the number of lampreys passing barriers need to be monitored to ensure spawning grounds may be reached. Fish passes need to be introduced at locations where passage is not possible and lamprey-friendly designs needs to be implemented. The efficacy of these fish passes also needs to be assessed so that connectivity between designated SACs can be assured and gene flow is possible with isolated populations. However, although a network of SACs, connecting lamprey populations and safeguarding the connectivity and maintenance of specific habitats, seems like it should be adequate protection for the conservation of *L. fluviatilis* and *L. planeri*, adequate resources also need to be made available for the implementation of conservation legislation and these legal frameworks need to be enforced.

## 5.7 Recommendations for future research

There are a number of key areas on which future research efforts should focus. As outlined in Section 5.4, gathering fundamental information about lamprey populations on a catchment by catchment basis such as; distribution, estimation of population including

numbers of adults spawning each year; and the availability of larval and spawning habitat to ensure that more valid conservation strategies are implemented in the future. Further research is also needed to assess wider and longer-term impacts of hydropower schemes (e.g. habitat change, changes in species abundance or composition, and changes to overall flow regime) on lamprey populations and other fish communities.

As outlined previously, the longitudinal connectivity within rivers is also vital to the persistence of lampreys, and other riverine species with a variety of habitat needs. Barriers to migration within rivers that are used by spawning adult lamprey should be identified and mitigation put into place in the form of lamprey-friendly fish-passes allowing access to further spawning sites. Low-gradient vertical slot, or nature-like fishways are likely to be the most efficient for *L. fluviatilis* passage, as well as allowing passage for other riverine taxa (Rodríguez *et al.* 2006; Calles & Greenberg 2007; Pratt *et al.* 2009; Noonan *et al.* 2012; Foulds & Lucas 2013). However, further research is needed to assess the passage efficiency of these fishways for lampreys.

As outlined in Section 5.4, eDNA (Thomsen *et al.* 2012) has huge potential in the monitoring of lamprey populations and other fish species of conservation concern. This method need to be assessed for its potential, and accuracy, as a management tool. Further investigation into the genetics of *L. planeri* and *L. fluviatilis* populations by next generation sequencing methods, using whole genomes, should also be a priority both from an evolutionary, and a conservation management perspective. Although costly at this point in time, next generation sequencing should allow more inference into dynamics of this species-pair, allowing for more effective management strategies to be put into place.

### 5.7.1 The use of larval pheromones as a conservation tool

As outlined in Chapter 1 (Section 1.5) migratory sea lamprey (*Petromyzon marinus*) use a pheromone mixture released by larval lampreys to locate suitable spawning sites (Sorensen *et al.* 2005). This mixture is comprised of three steroids: petromyzonol sulphate (PS), petromyzonamine disulfate (PADS), and petromyzosterol disulfate (PSDS) (Li *et al.* 1995; Polkinghorne *et al.* 2001; Fine & Sorensen 2005; Sorensen *et al.* 2005). Substantial concentrations of PS and ACA have already been measured in the larval tissues of two other lamprey species, *Entosphenus tridentatus* (an anadromous, parasitic species) and *Lampetra richardsoni* (a non-parasitic, freshwater-resident species) (Yun *et al.* 2003a). Migrating adult *E. tridentatus* have also been found to show sensitivity to the larval lamprey bile acid PS (and also a component of the sea lamprey sex pheromone; 3-keto petromyzonol sulfate) but apparently differ in having a longer period of sensitivity than *P. marinus* (Robinson et al. 2009). The initial findings of Gaudron and Lucas (2006) have strongly suggested that adult *L. fluviatilis* in the early migratory phase are attracted to conspecific larval odour. It has, however, not yet been established whether PS and ACA are being produced by *L. fluviatilis,* or *L. planeri,* larvae and whether the migratory adults of both species will respond to pheromones produced by heterospecific larvae.

Other studies have shown that adult lamprey attraction to larval lamprey pheromones tends to lack species specificity (Fine *et al.* 2004; Baker *et al.* 2009; Yun *et al.* 2011). Therefore, it is highly probable, that within a closely related species-pair such as *L. fluviatilis* and *L. planeri*, that the odours released by juveniles from each species are likely to be attractive to adults of both *L. planeri* and *L. fluviatilis*. This could prove useful in assuring the connectivity of habitats, and SACs, if barriers within a catchment were removed as pheromones could be applied to assist the occurrence of communal spawning in multi-species aggregations. For

instance, if existing barriers were removed downstream of isolated populations of *L. planeri*, the heterospecificity of larval pheromones would mean that adult *L. fluviatilis* may be encouraged to migrate to the *L. planeri* spawning sites, and gene flow with these populations may be resumed. In this way, pheromones could also be applied in trying to increase lamprey populations by: using pheromones to attract adults to suitable spawning streams, to direct migrants away from hydropower facilities that are likely to hinder migration, or to enhance spawning runs where lamprey populations already exist (Close *et al.* 2002; Yun *et al.* 2003b).

The cues migrating lampreys react to when selecting which odour plumes to follow is also vital when considering the application of pheromones in conservation management. In laboratory tests, it has been shown that migrating lampreys strongly avoid swimming in areas of the channel devoid of larval odour (Wagner *et al.* 2009). Due to larval pheromones playing a key role in the attraction of maturing adults and their retention in rivers (Vrieze *et al.* 2010) the application of larval pheromones to channels devoid of larval odour, but rich in spawning and larval habitat, could result in greater recruitment. Chemical cues, therefore, have the potential to play an important role in the reestablishment, or redirection of populations affected by anthropogenic changes, into suitable spawning habitat. Due to *P. marinus*, *L. fluviatilis*, and *L. planeri* occurring sympatrically within the British Isles and Western Europe, it would be of great value, both from an evolutionary and conservation perspective, to establish whether there is an overlap in pheromone production and reception within these three species. If PS is produced by all larval lampreys, and is attractive to migratory adults of any lamprey species, this application has a wider scope within lamprey conservation worldwide. Therefore, future research should aim to

determine whether odour produced by larval *L. planeri, L. fluviatilis,* and *P. marinus* are all attractive to migratory adults of these species.

The quantification of petromyzonol sulphate (PS), released by larval lampreys, within rivers has also been suggested as a management tool (Stewart & Baker 2012) similar to eDNA. Stewart *et al.* (2011) recently reported a new, highly sensitive method for the rapid identification and quantification of these pheromones in river water samples using a procedure based on pre-concentration, followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. The southern pouched lamprey (*Geotria australis*) has shown to be sensitive to PS released by larval lampreys (Baker *et al.* 2009). Using the recently developed sensitive mass spectrometry method (Stewart *et al.* 2011), Stewart and Baker (2012) compared passive sampling techniques against more traditional active water sampling as methods for estimating lamprey populations in local streams using PS concentrations. Passive sampling provided quantitative data for PS from all sites surveyed. Conversely, active sampling returned only one positive result out of 19 samples, and with a method detection limit of $2.5\times10^{14}$ M, this suggests that concentrations of PS in these streams are either extremely low or variable. Therefore, Stewart and Baker (2012) identified a combination of passive sampling and triple quadruple mass spectrometry as a promising tool for monitoring of *G. australis* in New Zealand streams. Earlier studies have shown that PS degrades rapidly in raw water due to microbial breakdown, with a half-life of 1.5 days (Polkinghorne *et al.* 2001) which would need to be taken into account if passive sampling was to be utilised, and measures taken to ensure PS does not degrade before samples are analysed. This method also holds potential for sampling *L. fluviatilis* within rivers, and perhaps also *L. planeri* if they too release PS.

### 5.7.2 Social attitudes and public perception of lampreys

Public perception assessments have historically been biased towards mammals (McKinstry & Anderson 1999; Kaczensky *et al.* 2004; Meadow *et al.* 2005) driven by perceived threats to human safety (e.g. bears), livestock (e.g. wolves) and land use change (e.g. beavers). However, public attitudes have also been examined during the reintroduction of burbot *Lota lota* to the UK (Worthington *et al.* 2010), and in the management of sea lamprey (*P. marinus*) in North America (Meyer Resources Inc. 1989). The changing societal perception of sharks has played an important role, not only in the use of the ocean by the public, but also in relation to research, management and conservation. In a study by Simpfendorfer *et al.* (2011), the change in social perception of sharks was examined over a period of several decades. The recognition that sharks can also provide financial benefits to communities beyond those provided by fishing has contributed to the perception of the value of sustaining the world's sharks. The reasons for this change in perception are poorly understood, but no doubt have been contributed to by a better understanding of sharks and the oceans. The change in perception of sharks has had several significant implications for scientific research such as an increase in the resources available to support research, an increase in the amount of research conducted, an increase in people willing to undertake this research, especially at the student level, and also the growth in the number of societies dedicated to the science, conservation and management of sharks.

Exploring public opinion and attitudes towards the conservation of lampreys can contribute to effective management of their populations, and examine how a change in the public perception can affect the impetus for this action. Traditional research topics are insufficient to fully implement conservation plans because managing resources is as much about understanding the resource as it is about managing the people who exploit it

(Hilborn 2007). Thus, research to understand the values, behaviours, attitudes and actions of the people, industries and communities that directly (or indirectly) have a vested interest in lampreys, and the aquatic ecosystem, will be equally as important. It is of particular importance to assess people's values in this way as they are known to shape attitudes, norms and behaviour in wildlife management (Bright *et al.* 2000; Manfredo & Dayer 2004). Similarly, there is a need to develop partnerships with stakeholders in affected ecosystems to strengthen and implement fish conservation activities, and develop mechanisms to influence other players. To achieve this, scientists must expand their range of activities from monitoring and reporting the status of endangered species to more influential and preventative work (Collares-Pereira & Cowx 2004).

The main concern for lamprey conservation should be the inclusion of qualitative studies evaluating the social implications of lamprey conservation and outlining the general public perception and stakeholder attitudes towards lampreys. This should outline a strategy to create more awareness of lamprey conservation issues and facilitate active engagement of regulatory bodies and government. The general populace have poor awareness of the problems facing lampreys and freshwater fishes in general, thus greater opportunity should be made of their willingness to support conservation campaigns by promoting education and extension programmes (Cambray & Pister 2002). As such, public backing of conservation incentives should also create a feedback effect in which more interest will be taken in the conservation of lampreys and thus more funding will become available for research, ultimately enabling more effective conservation initiatives through better understanding of these animals. This is an area of research that has lagged well behind that of the biology and ecology (Simpfendorfer *et al.* 2011) but seems to hold an equivalent weight in the progression and success of conservation management. It therefore seems

fundamental to adjoin the social research 'string' to our conservation 'bow', which may bring us closer to hitting the ultimate target - the protection of lamprey populations and the maintenance of the fragile balance that exists within freshwater ecosystems.

The lamprey is our elder, without him the circle of life is broken.

—*Elmer Crow Jr.,* Vice Chair, Nez Perce Fish and Wildlife Committe

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**Appendix A** Table outlining diversity indices for microsatellite loci by population, and by locus. Where Lp = *Lampetra planeri*, Lf = *Lampetra fluviatilis,* and Lf Res = freshwater-resident population of *L. fluviatilis*. *Na* = number of alleles, *Ar* = allelic richness, *Ho* = observed homozygosity, *He* = expected homozygosity, *p-value* = indicates significance of deviation from Hardy-Weinberg equilibrium (after Bonferroni correction significance level was adjusted to $P < 0.000226$. Any P-values still significant after this are highlighted in a grey box). *Fis* = inbreeding coefficient and *Null alleles* = presence of any null alleles that are highlighted in a green box and shown as estimated allele frequency using the Oosterhout algorithm

| | Locus | Lp_003 | Lp_009 | Lamper_1 | Lamper _2 | Lri_5 | Lp_027 | Lp_028 | Lp_046 | Lamper _3 | Lp_006 | Lp_045 | lp_018 | Lamper_4 | All |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wear (Lf) | ***Na*** | 2.000 | 4.000 | 12.000 | 18.000 | 3.000 | 4.000 | 5.000 | 3.000 | 4.000 | 2.000 | 4.000 | 5.000 | 17.000 | |
| | ***Ar*** | 1.998 | 3.204 | 7.859 | 10.872 | 2.849 | 3.863 | 4.413 | 2.987 | 2.826 | 1.898 | 3.520 | 4.557 | 11.353 | |
| | ***Ho*** | 0.314 | 0.639 | 0.833 | 0.829 | 0.500 | 0.735 | 0.806 | 0.583 | 0.583 | 0.111 | 0.583 | 0.778 | 0.861 | |
| | ***He*** | 0.342 | 0.593 | 0.803 | 0.909 | 0.542 | 0.683 | 0.683 | 0.521 | 0.504 | 0.155 | 0.597 | 0.665 | 0.921 | |
| | ***p-value*** | 0.631 | 0.193 | 0.512 | 0.034 | 0.156 | 0.947 | 0.384 | 0.887 | 0.281 | 0.203 | 0.925 | 0.030 | 0.282 | |
| | ***Fis*** | 0.081 | -0.079 | -0.039 | 0.090 | 0.078 | -0.077 | -0.182 | -0.122 | -0.159 | 0.286 | 0.023 | -0.173 | 0.066 | -0.040 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| Wear (Lp) | ***Na*** | 2.000 | 3.000 | 2.000 | 11.000 | 3.000 | 3.000 | 3.000 | 2.000 | 2.000 | 2.000 | 2.000 | 3.000 | 7.000 | |
| | ***Ar*** | 2.000 | 2.995 | 1.619 | 7.979 | 2.374 | 2.379 | 2.480 | 2.000 | 2.000 | 1.769 | 2.000 | 2.619 | 6.117 | |
| | ***Ho*** | 0.621 | 0.586 | 0.069 | 0.724 | 0.379 | 0.448 | 0.207 | 0.448 | 0.310 | 0.103 | 0.731 | 0.724 | 0.724 | |
| | ***He*** | 0.508 | 0.638 | 0.068 | 0.789 | 0.319 | 0.399 | 0.194 | 0.390 | 0.390 | 0.100 | 0.509 | 0.541 | 0.742 | |
| | ***p-value*** | 0.278 | 0.025 | 1.000 | 0.622 | 0.631 | 0.730 | 1.000 | 0.636 | 0.338 | 1.000 | 0.046 | 0.046 | 0.541 | |
| | ***Fis*** | -0.226 | 0.083 | -0.018 | 0.084 | -0.194 | -0.127 | -0.070 | -0.152 | 0.208 | -0.037 | -0.448 | -0.346 | 0.025 | -0.095 |
| | ***Null alleles*** | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Dee (Lf)** | ***Na*** | 3.000 | 4.000 | 10.000 | 21.000 | 3.000 | 5.000 | 4.000 | 3.000 | 4.000 | 2.000 | 5.000 | 5.000 | 24.000 | |
| | ***Ar*** | 2.557 | 3.539 | 6.864 | 11.688 | 2.811 | 4.317 | 3.969 | 2.993 | 2.892 | 1.878 | 3.608 | 4.148 | 12.345 | |
| | ***Ho*** | 0.455 | 0.515 | 0.727 | 0.818 | 0.364 | 0.607 | 0.727 | 0.697 | 0.515 | 0.152 | 0.419 | 0.727 | 0.909 | |
| | ***He*** | 0.386 | 0.570 | 0.748 | 0.897 | 0.459 | 0.687 | 0.737 | 0.615 | 0.551 | 0.142 | 0.599 | 0.574 | 0.916 | |
| | ***p-value*** | 0.173 | 0.517 | 0.207 | 0.553 | 0.257 | 0.339 | 0.014 | 0.662 | 0.863 | 1.000 | 0.136 | 0.429 | 0.184 | |
| | ***Fis*** | -0.181 | 0.098 | 0.028 | 0.090 | 0.210 | 0.118 | 0.013 | -0.136 | 0.065 | -0.067 | 0.303 | -0.273 | 0.008 | 0.013 |
| | ***Null alleles*** | | | | | | | | | | | 0.135 | | | |
| **Dee (Lp)** | ***Na*** | 2.000 | 4.000 | 9.000 | 8.000 | 3.000 | 4.000 | 4.000 | 3.000 | 2.000 | 3.000 | 3.000 | 3.000 | 12.000 | |
| | ***Ar*** | 1.994 | 3.323 | 5.977 | 5.710 | 2.999 | 3.970 | 3.748 | 2.698 | 2.000 | 1.969 | 2.965 | 2.363 | 8.030 | |
| | ***Ho*** | 0.267 | 0.533 | 0.700 | 0.714 | 0.700 | 0.667 | 0.600 | 0.300 | 0.367 | 0.100 | 0.556 | 0.400 | 0.733 | |
| | ***He*** | 0.282 | 0.458 | 0.714 | 0.686 | 0.654 | 0.674 | 0.613 | 0.269 | 0.503 | 0.098 | 0.570 | 0.332 | 0.840 | |
| | ***p-value*** | 1.000 | 1..000 | 0.233 | 0.292 | 1.000 | 0.393 | 0.766 | 1.000 | 0.159 | 1.000 | 1.000 | 0.633 | 0.010 | |
| | ***Fis*** | 0.057 | -0.169 | 0.020 | -0.042 | -0.072 | 0.012 | 0.022 | -0.115 | 0.275 | -0.024 | 0.025 | -0.210 | 0.128 | -0.016 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Derwent (Lf)** | ***Na*** | 3.000 | 4.000 | 9.000 | 19.000 | 4.000 | 4.000 | 5.000 | 3.000 | 5.000 | 3.000 | 4.000 | 6.000 | 20.000 | |
| | ***Ar*** | 2.617 | 3.297 | 6.661 | 12.413 | 3.297 | 3.982 | 4.385 | 2.998 | 3.336 | 2.215 | 3.372 | 4.657 | 12.018 | |
| | ***Ho*** | 0.448 | 0.586 | 0.586 | 0.846 | 0.483 | 0.852 | 0.786 | 0.567 | 0.567 | 0.167 | 0.519 | 0.607 | 0.800 | |
| | ***He*** | 0.387 | 0.595 | 0.717 | 0.932 | 0.528 | 0.679 | 0.760 | 0.631 | 0.570 | 0.158 | 0.591 | 0.718 | 0.920 | |
| | ***p-value*** | 0.294 | 0.152 | 0.039 | 0.035 | 0.752 | 0.588 | 0.963 | 0.736 | 0.445 | 1.000 | 0.240 | 0.535 | 0.104 | |

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| | ***Fis*** | -0.161 | 0.016 | 0.185 | 0.094 | 0.086 | -0.262 | -0.035 | 0.103 | 0.006 | -0.058 | 0.124 | 0.157 | 0.132 | -0.004 |
| | ***Null alleles*** | | | | | | | | | | | | | 0.058 | |
| **Derwent (Lp)** | ***Na*** | 4.000 | 3.000 | 6.000 | 11.000 | 2.000 | 3.000 | 4.000 | 4.000 | 3.000 | 3.000 | 3.000 | 4.000 | 15.000 | |
| | ***Ar*** | 2.687 | 2.724 | 4.012 | 7.066 | 2.000 | 2.999 | 3.983 | 3.343 | 2.344 | 2.068 | 2.753 | 2.687 | 10.417 | |
| | ***Ho*** | 0.500 | 0.531 | 0.656 | 0.781 | 0.387 | 0.767 | 0.750 | 0.656 | 0.375 | 0.125 | 0.533 | 0.625 | 1.000 | |
| | ***He*** | 0.479 | 0.487 | 0.549 | 0.814 | 0.503 | 0.658 | 0.738 | 0.670 | 0.469 | 0.121 | 0.481 | 0.479 | 0.909 | |
| | ***p-value*** | 1.000 | 0.862 | 0.126 | 0.051 | 0.279 | 0.293 | 0.135 | 0.513 | 0.391 | 1.000 | 0.727 | 0.181 | **0.000** | |
| | ***Fis*** | -0.045 | -0.093 | -0.200 | 0.041 | 0.234 | -0.169 | -0.017 | 0.020 | 0.203 | -0.038 | -0.111 | -0.312 | -0.102 | -0.058 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Nidd (Lf)** | ***Na*** | 3.000 | 4.000 | 9.000 | 21.000 | 4.000 | 5.000 | 4.000 | 4.000 | 4.000 | 2.000 | 3.000 | 4.000 | 20.000 | |
| | ***Ar*** | 2.367 | 3.742 | 6.481 | 13.072 | 3.120 | 4.338 | 3.912 | 3.599 | 3.120 | 1.967 | 2.908 | 3.948 | 12.691 | |
| | ***Ho*** | 0.533 | 0.533 | 0.700 | 0.867 | 0.600 | 0.800 | 0.800 | 0.667 | 0.367 | 0.167 | 0.633 | 0.700 | 0.900 | |
| | ***He*** | 0.437 | 0.666 | 0.695 | 0.941 | 0.490 | 0.682 | 0.669 | 0.663 | 0.571 | 0.210 | 0.559 | 0.688 | 0.934 | |
| | ***p-value*** | 0.553 | 0.048 | 0.161 | 0.007 | 0.192 | 0.627 | 0.306 | 0.883 | 0.065 | 0.326 | 0.433 | 0.027 | 0.095 | |
| | ***Fis*** | -0.226 | 0.201 | -0.007 | 0.080 | -0.230 | -0.177 | -0.199 | -0.006 | 0.362 | 0.208 | -0.136 | -0.018 | 0.037 | -0.008 |
| | ***Null alleles*** | | | | | | | | | 0.170 | | | | | |
| **Nidd (Lp)** | ***Na*** | 2.000 | 4.000 | 2.000 | 8.000 | 2.000 | 4.000 | 4.000 | 3.000 | 2.000 | 1.000 | 4.000 | 4.000 | 7.000 | |
| | ***Ar*** | 2.000 | 3.301 | 1.999 | 6.033 | 2.000 | 3.215 | 3.312 | 2.989 | 2.000 | 1.000 | 3.450 | 3.829 | 5.057 | |
| | ***Ho*** | 0.867 | 0.367 | 0.433 | 0.655 | 0.367 | 0.500 | 0.633 | 0.533 | 0.467 | mono | 0.533 | 0.733 | 0.700 | |

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| | ***He*** | 0.499 | 0.325 | 0.345 | 0.649 | 0.494 | 0.581 | 0.606 | 0.595 | 0.452 | mono | 0.489 | 0.633 | 0.699 | |
| | ***p-value*** | **0.000** | 1.000 | 0.291 | 0.410 | 0.255 | 0.613 | 0.360 | 0.735 | 1.000 | mono | 0.399 | 0.525 | 0.382 | |
| | ***Fis*** | -0.758 | -0.131 | -0.261 | -0.009 | 0.262 | 0.142 | -0.046 | 0.105 | -0.033 | N.A. | -0.093 | -0.162 | -0.001 | -0.071 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Ure (Lf)** | ***Na*** | 3.000 | 3.000 | 8.000 | 24.000 | 2.000 | 4.000 | 4.000 | 3.000 | 4.000 | 2.000 | 3.000 | 5.000 | 23.000 | |
| | ***Ar*** | 2.379 | 2.995 | 6.136 | 13.917 | 2.000 | 3.981 | 3.936 | 2.984 | 2.759 | 1.984 | 2.918 | 4.582 | 13.426 | |
| | ***Ho*** | 0.586 | 0.621 | 0.759 | 0.966 | 0.276 | 0.793 | 0.586 | 0.586 | 0.552 | 0.276 | 0.552 | 0.828 | 0.828 | |
| | ***He*** | 0.482 | 0.617 | 0.733 | 0.942 | 0.436 | 0.724 | 0.673 | 0.615 | 0.528 | 0.242 | 0.578 | 0.675 | 0.938 | |
| | ***p-value*** | 0.498 | 0.307 | 0.721 | 0.822 | 0.077 | 0.955 | 0.558 | 0.168 | 0.810 | 1.000 | 0.409 | 0.056 | 0.083 | |
| | ***Fis*** | -0.221 | -0.006 | -0.035 | -0.026 | 0.371 | -0.097 | 0.131 | 0.047 | -0.047 | -0.143 | 0.046 | -0.232 | 0.120 | -0.003 |
| | ***Null alleles*** | | | | | | | | | | | | | 0.053 | |
| **Ure (Lp)** | ***Na*** | 2.000 | 3.000 | 4.000 | 10.000 | 2.000 | 3.000 | 3.000 | 4.000 | 3.000 | 2.000 | 3.000 | 4.000 | 8.000 | |
| | ***Ar*** | 2.000 | 2.847 | 3.734 | 6.968 | 2.000 | 3.000 | 2.845 | 3.355 | 2.967 | 1.849 | 2.603 | 3.328 | 5.324 | |
| | ***Ho*** | 0.600 | 0.467 | 0.533 | 0.867 | 0.367 | 0.500 | 0.500 | 0.600 | 0.667 | 0.133 | 0.500 | 0.433 | 0.467 | |
| | ***He*** | 0.472 | 0.425 | 0.640 | 0.835 | 0.481 | 0.678 | 0.406 | 0.572 | 0.605 | 0.127 | 0.520 | 0.458 | 0.595 | |
| | ***p-value*** | 0.233 | 0.825 | 0.001 | 0.898 | 0.250 | 0.027 | 0.532 | 0.900 | 0.143 | 1.000 | 0.030 | 0.235 | 0.039 | |
| | ***Fis*** | -0.276 | -0.100 | 0.169 | -0.039 | 0.240 | 0.266 | -0.236 | -0.049 | -0.104 | -0.055 | 0.040 | 0.055 | 0.218 | 0.027 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Swale (Lf)** | ***Na*** | 4.000 | 3.000 | 8.000 | 27.000 | 4.000 | 5.000 | 5.000 | 4.000 | 4.000 | 4.000 | 4.000 | 6.000 | 20.000 | |

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| | ***Ar*** | 2.687 | 2.984 | 6.201 | 14.325 | 2.687 | 4.297 | 4.299 | 3.338 | 2.687 | 2.890 | 3.529 | 4.574 | 12.466 | |
| | ***Ho*** | 0.438 | 0.688 | 0.750 | 0.906 | 0.406 | 0.750 | 0.563 | 0.594 | 0.750 | 0.344 | 0.563 | 0.688 | 0.844 | |
| | ***He*** | 0.412 | 0.615 | 0.697 | 0.949 | 0.441 | 0.727 | 0.668 | 0.601 | 0.531 | 0.302 | 0.607 | 0.676 | 0.931 | |
| | ***p-value*** | 0.519 | 0.700 | 0.378 | 0.116 | 0.822 | 0.057 | 0.072 | 0.566 | 0.013 | 1.000 | 0.556 | 0.082 | 0.158 | |
| | ***Fis*** | -0.064 | -0.121 | -0.077 | 0.046 | 0.081 | -0.032 | 0.160 | 0.012 | -0.423 | -0.140 | 0.074 | -0.018 | 0.095 | -0.016 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Lomond (Lf)** | ***Na*** | 2.000 | 4.000 | 7.000 | 16.000 | 3.000 | 4.000 | 5.000 | 3.000 | 4.000 | 4.000 | 3.000 | 4.000 | 17.000 | |
| | ***Ar*** | 1.996 | 3.693 | 5.602 | 11.520 | 2.998 | 3.870 | 4.471 | 2.961 | 2.957 | 2.908 | 2.998 | 3.467 | 12.135 | |
| | ***Ho*** | 0.333 | 0.583 | 0.750 | 0.833 | 0.583 | 0.609 | 0.696 | 0.458 | 0.478 | 0.375 | 0.500 | 0.565 | 0.875 | |
| | ***He*** | 0.284 | 0.577 | 0.731 | 0.919 | 0.621 | 0.623 | 0.750 | 0.483 | 0.519 | 0.324 | 0.637 | 0.557 | 0.932 | |
| | ***p-value*** | 1.000 | 0.383 | 0.676 | 0.003 | 0.028 | 0.483 | 0.034 | 0.740 | 0.669 | 1.000 | 0.123 | 0.695 | 0.139 | |
| | ***Fis*** | -0.179 | -0.011 | -0.026 | 0.095 | 0.063 | 0.024 | 0.074 | 0.052 | 0.080 | -0.160 | 0.219 | -0.014 | 0.062 | 0.028 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Lomond (Lf Res)** | ***Na*** | 2.000 | 3.000 | 5.000 | 11.000 | 3.000 | 5.000 | 2.000 | 4.000 | 3.000 | 3.000 | 3.000 | 5.000 | 10.000 | |
| | ***Ar*** | 2.000 | 2.836 | 4.191 | 8.091 | 2.739 | 4.550 | 2.000 | 2.942 | 2.355 | 2.900 | 2.977 | 3.777 | 7.472 | |
| | ***Ho*** | 0.677 | 0.710 | 0.774 | 0.667 | 0.710 | 0.677 | 0.226 | 0.645 | 0.581 | 0.419 | 0.484 | 0.613 | 0.742 | |
| | ***He*** | 0.455 | 0.565 | 0.722 | 0.829 | 0.554 | 0.694 | 0.373 | 0.517 | 0.428 | 0.359 | 0.538 | 0.500 | 0.848 | |
| | ***p-value*** | 0.006 | 0.014 | 0.838 | 0.008 | 0.003 | 0.027 | 0.042 | 0.140 | 0.087 | 0.775 | 0.577 | 0.135 | **0.000** | |
| | ***Fis*** | -0.500 | -0.261 | -0.074 | 0.199 | -0.288 | 0.025 | 0.398 | -0.254 | -0.365 | -0.171 | 0.102 | -0.231 | 0.127 | -0.080 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Lomond (Lp)** | ***Na*** | 2.000 | 3.000 | 9.000 | 21.000 | 3.000 | 5.000 | 4.000 | 3.000 | 3.000 | 3.000 | 4.000 | 5.000 | 17.000 | |
| | ***Ar*** | 2.000 | 2.999 | 6.392 | 13.069 | 2.997 | 4.809 | 3.885 | 2.849 | 2.306 | 2.747 | 3.745 | 4.624 | 11.112 | |
| | ***Ho*** | 0.543 | 0.667 | 0.667 | 0.882 | 0.639 | 0.778 | 0.722 | 0.528 | 0.472 | 0.306 | 0.556 | 0.750 | 0.889 | |
| | ***He*** | 0.448 | 0.645 | 0.722 | 0.937 | 0.601 | 0.712 | 0.603 | 0.542 | 0.460 | 0.274 | 0.536 | 0.742 | 0.908 | |
| | ***p-value*** | 0.263 | 0.086 | 0.180 | 0.545 | 0.834 | 0.667 | 0.150 | 0.630 | 0.503 | 1.000 | 0.918 | 0.012 | 0.708 | |
| | ***Fis*** | -0.217 | -0.034 | 0.078 | 0.059 | -0.065 | -0.094 | -0.201 | 0.026 | -0.028 | -0.116 | -0.038 | -0.011 | 0.022 | -0.042 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Bann (Lf Res)** | ***Na*** | 3.000 | 2.000 | 4.000 | 15.000 | 3.000 | 4.000 | 3.000 | 3.000 | 4.000 | 1.000 | 3.000 | 4.000 | 9.000 | |
| | ***Ar*** | 2.394 | 2.000 | 3.603 | 10.835 | 2.691 | 3.977 | 2.712 | 2.988 | 3.458 | 1.000 | 2.456 | 3.885 | 7.724 | |
| | ***Ho*** | 0.200 | 0.520 | 0.600 | 0.880 | 0.560 | 0.750 | 0.500 | 0.520 | 0.667 | mono | 0.250 | 0.625 | 0.917 | |
| | ***He*** | 0.220 | 0.393 | 0.588 | 0.908 | 0.528 | 0.689 | 0.551 | 0.598 | 0.680 | mono | 0.345 | 0.629 | 0.871 | |
| | ***p-value*** | 0.110 | 0.145 | 0.955 | 0.252 | 1.000 | 0.576 | 0.841 | 0.483 | 0.694 | mono | 0.322 | 0.248 | 0.237 | |
| | ***Fis*** | 0.091 | -0.333 | -0.021 | 0.031 | -0.062 | -0.091 | 0.094 | 0.133 | 0.020 | N.A. | 0.279 | 0.006 | -0.054 | -0.021 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Scheldt (Lf)** | ***Na*** | 3.000 | 4.000 | 8.000 | 19.000 | 3.000 | 4.000 | 4.000 | 3.000 | 5.000 | 2.000 | 3.000 | 5.000 | 23.000 | |
| | ***Ar*** | 2.531 | 3.308 | 6.248 | 11.520 | 2.533 | 3.931 | 3.989 | 2.959 | 3.380 | 1.984 | 2.314 | 4.668 | 12.676 | |
| | ***Ho*** | 0.429 | 0.543 | 0.771 | 0.824 | 0.429 | 0.629 | 0.714 | 0.543 | 0.571 | 0.229 | 0.600 | 0.829 | 0.886 | |

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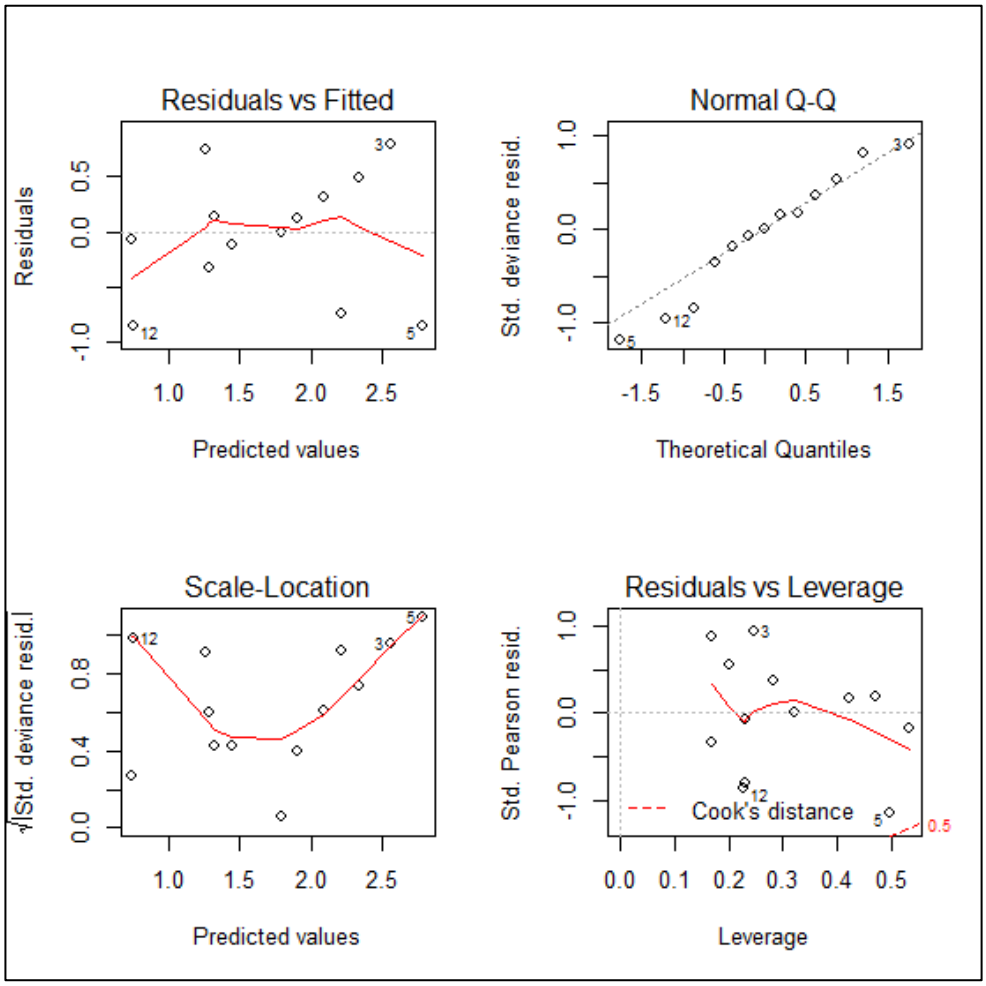
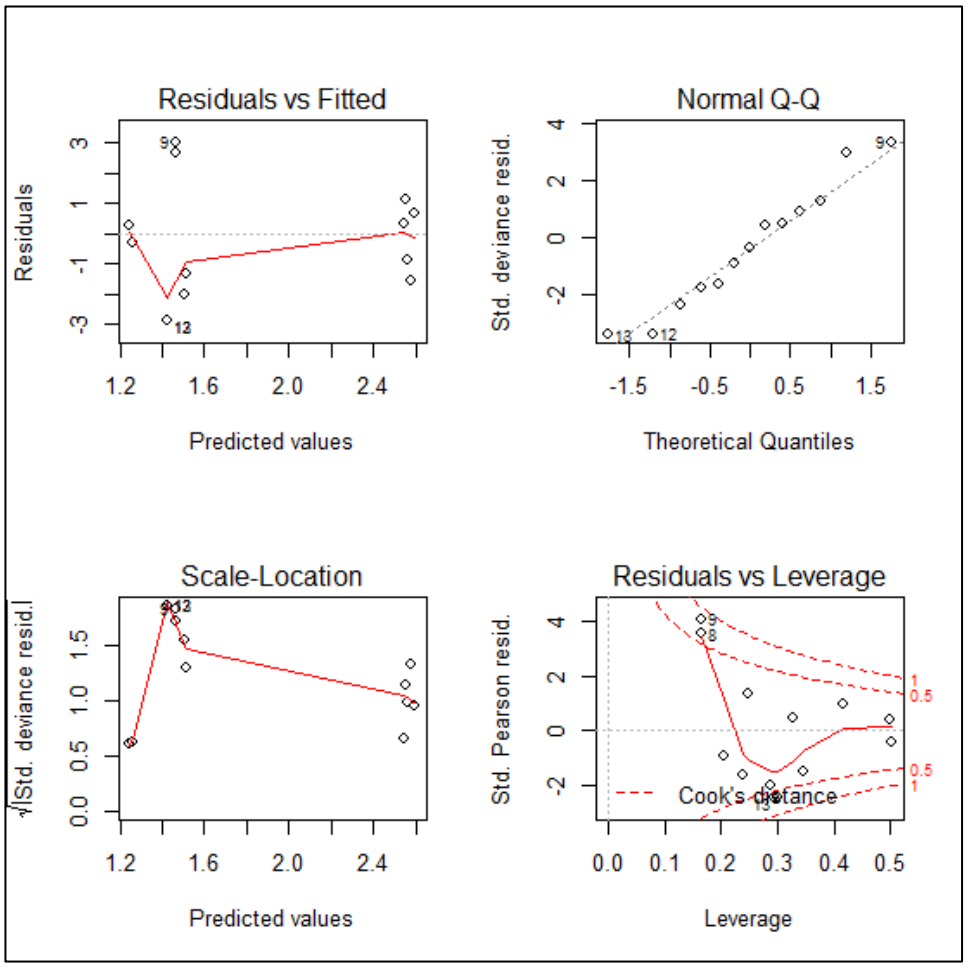
| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***He*** | 0.386 | 0.618 | 0.708 | 0.910 | 0.442 | 0.675 | 0.732 | 0.510 | 0.574 | 0.248 | 0.519 | 0.688 | 0.931 | |
| | ***p-value*** | 1.000 | 0.747 | 0.939 | 0.386 | 1.000 | 0.570 | 0.171 | 0.793 | 1.000 | 0.527 | 0.403 | 0.298 | 0.772 | |
| | ***Fis*** | -0.114 | 0.123 | -0.091 | 0.097 | 0.030 | 0.070 | 0.024 | -0.065 | 0.005 | 0.081 | -0.159 | -0.208 | 0.049 | -0.010 |
| | ***Null alleles*** | | | | | | | | | | | | | | |
| **Trent (Lf)** | ***Na*** | 2.000 | 4.000 | 10.000 | 24.000 | 3.000 | 5.000 | 5.000 | 4.000 | 5.000 | 4.000 | 3.000 | 5.000 | 21.000 | |
| | ***Ar*** | 2.000 | 3.547 | 5.886 | 13.261 | 2.811 | 4.296 | 4.545 | 3.322 | 3.377 | 3.141 | 2.922 | 4.022 | 12.863 | |
| | ***Ho*** | 0.455 | 0.636 | 0.606 | 0.909 | 0.606 | 0.667 | 0.818 | 0.758 | 0.576 | 0.394 | 0.636 | 0.667 | 0.879 | |
| | ***He*** | 0.461 | 0.628 | 0.630 | 0.933 | 0.472 | 0.689 | 0.756 | 0.625 | 0.576 | 0.418 | 0.558 | 0.582 | 0.937 | |
| | ***p-value*** | 1.000 | 0.179 | 0.187 | 0.727 | 0.220 | 0.202 | 0.094 | 0.188 | 0.212 | 0.664 | 0.625 | 0.205 | 0.473 | |
| | ***Fis*** | 0.014 | -0.013 | 0.038 | 0.026 | -0.289 | 0.032 | -0.084 | -0.216 | 0.001 | 0.059 | -0.144 | -0.148 | 0.063 | -0.042 |
| | ***Null alleles*** | | | | | | | | | | | | | | |

**Appendix B** The model check plots for GLM's for the **a)** ammocoete catch data and **b)** transformer catch data. Too few data are available to effectively interpret whether or not any strong patterns occur within the plots for the most part, however, the Q-Q plots in both **a)** and **b)** indicate that the error was normally distributed indicating that the models were performing adequately.

**a)**



**b)**


Residuals vs Fitted
Residuals
Predicted values
Normal Q-Q
Std. deviance resid.
Theoretical Quantiles
Scale-Location
√|Std. deviance resid.|
Predicted values
Residuals vs Leverage
Std. Pearson resid.
Cook's distance
Leverage